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BEHAVIOR OF MERCURY, CHROMIUM, AND CADMIUM
IN AQUATIC SYSTEMS

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FOREWORD

Environmental protection efforts are increasingly directed towards preventing adverse health and ecological effects associated with specific compounds of natural or human origin. As part of this Laboratory's research on the occurrence, movement, transformation, impact, and control of environmental contaminants, the Environmental Systems Branch studies the environmental transport, transformation, degradation, and impact of pollutants or other materials in soil and water.

This report examines the sequence of reactions that control the fate and possible transformations of mercury, cadmium, and chromium in aquatic environments. Because of the widespread industrial uses of these toxic substances, it is particularly important to understand their uptake and release patterns in biota.

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ABSTRACT

This report is concerned with determining the fate and possible transformations of mercury, cadmium, and chromium in freshwater sediment-water environments. Mercury and cadmium show a high affinity for natural organic (humic and fulvic) material. Organic material may also cause or catalyze the reduction of ionic mercury to elemental mercury. The rate of release of elemental mercury from lake sediments depends on both the amount and form of the organic material present, the Eh and pH of the environment.

Under continuous exposure, elemental mercury is readily accumulated by fish (*Gambusia*) at a rate comparable with ionic mercury. However, uptake is five times greater than ionic mercury under periodic exposure conditions. The excretion rates of elemental mercury approximately equal ionic mercury.

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SECTION I

INTRODUCTION

This report is concerned with determining the fate of mercury, cadmium, and chromium in freshwater sediment-water environments. Addressed in particular are some of the problems in interpreting the contaminant-organic interaction, which at this phase boundary in the environment may be the most important sequence of reactions that control the fate of these particular elements.

MERCURY

There are three components of the mercury (Hg) cycle in freshwater systems, two of which are shown in Fig. 1: uptake of mercury from the water by the sediments and release of mercury from the sediments to the water. The third component, uptake of mercury by the biota, particularly fish, is examined in this report with particular reference to elemental (Hg^0) mercury. Uptake of mercury compounds from water by fish has been extensively studied (Burrows and Krenkel, 1973; Rucker and Amend, 1969; Schoper, 1974) and does not appear to be a limiting step in the mercury cycle. In fact, uptake by fish has been used as a measure of the release of mercury from sediments (Gillespie and Scott), 1971; Gillespie, 1972; Langley, 1973). Besides direct uptake, fish may also accumulate mercury through the food chain, although the importance of this pathway is unknown. Hannerz (quoted in Krenkel, 1973) found no correlation between trophic level and concentration of mercury, including in his samples various invertebrates and fish. He attributed this to the fact that mercury uptake depends on several factors, including temperature, metabolic rate, and feeding habits. Jernelov and Lann (1971) studied a freshwater food chain involving benthic organisms, bottom-feeding fish and a top predator, northern pike. The benthic animals reflected the concentration of mercury in the sediments in which they were living, while the fish apparently had a basic mercury concentration derived from their prey and the water. The mercury concentration in the bottom feeders was less than 25% attributable to the food chain (based on 10% to 15% uptake of mercury from food and 10% nutritional efficiency), while the same figure for pike was about 50%. In general, the concentration of mercury in fish and other biota depends to a variable but large extent on the release of mercury into the water from the sediments.

Cranston and Buckley (1972) found that dissolved mercury entering the water of the LaHave River in Nova Scotia was quickly taken up by suspended sediments and eventually removed to the bottom. Once mercury is associated with the sediment, several pathways are possible (Fig. 1). Where sulfide is

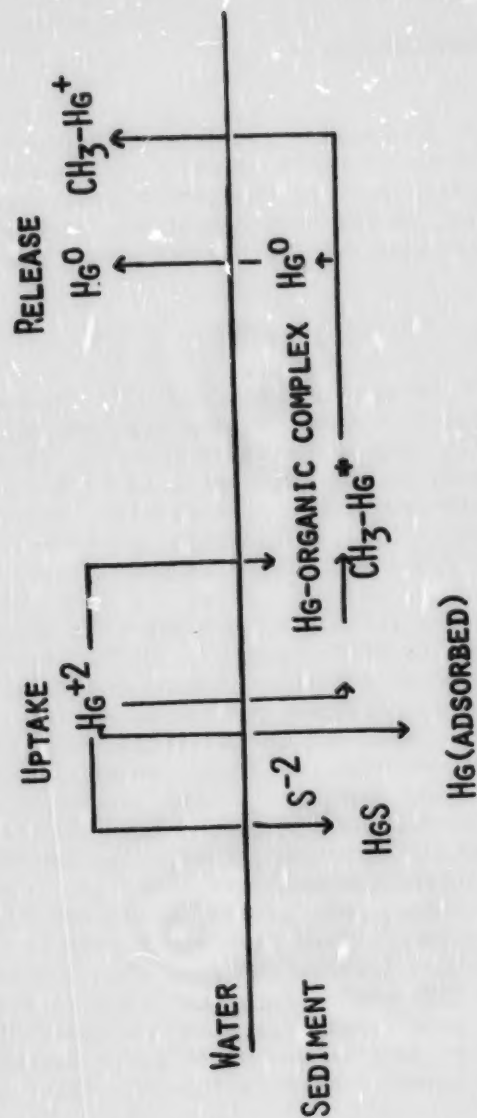


Figure 1. Mercury transformations in a water-sediment system. Not all possible reactions or intermediate compounds are shown here, and some of the processes may be reversible. All three forms of mercury shown in the water are readily taken up by the biota.

present, either free or in association with other elements, mercuric sulfide will be formed (Fagerström and Jernelöv, 1972). Even though the theoretical solubility product of mercuric sulfide (about 10^{-53}) is not strictly meaningful in a complex sedimentary system, it is a good indication of the relative immobility of mercury in this form (Gillespie and Scott, 1971; Fagerström and Jernelöv, 1971). Mercury may also be adsorbed by clays, sands, and oxides in the sediments, the stability of these complexes being dependent on the adsorbing material and various environmental conditions. Reimers and Krenkel (1974) found that clays were able to take up mercury to a greater extent than sand and that the capacity was approximately halved by a chloride concentration of 10,000 ppm. Rates of uptake without additional chloride, measured as microgram Hg/g sediment/min, were as follows:

Illite	>	Montmorillonite	>	Kaolinite	>	Sand
65.3		35.7		9.7		2.0

Desorption of mercury from sand was appreciable (about 10%) only at high chloride concentrations and was negligible in all cases from clays. Oxides of iron and manganese can also rapidly adsorb mercury, depending on pH and salt concentration, but desorption can be substantial, especially at high salinity (Jenne, 1970). The possibility also exists for mercury release under low redox conditions if the oxides are reduced and resolubilized (Krenkel, 1974).

In equilibrium with the various adsorbed forms of mercury present in sediments will be a small amount of Hg^{+2} dissolved in the interstitial water (Lindberg and Harris, 1974). Here, the possibility exists for bacterial formation of methylmercury, with subsequent release from the sediments. Westöb (1966), looking at the forms of mercury found in fish, noted that 75% or more of the total mercury was in the methyl form, $\text{CH}_3\text{-Hg}^+$. Since methylmercury was not a common pollutant, this indicated that a transformation of mercury compounds was occurring in the sediments. To test this, Jensen and Jernelöv (1969) added HgCl_2 to bottom sediments and analyzed for methylmercury. After seven days of incubation, a peak of about 110 ppb methylmercury was found in sediments to which 100 ppm HgCl_2 had been added. Smaller amounts of methylmercury were produced when either more or less HgCl_2 was added initially. Longer incubation of 100 ppm HgCl_2 showed that a plateau of methylmercury concentration occurred after about 6 days, with no increase over the next three weeks. They concluded that the methylmercury was formed by bacterial transformation of the added inorganic mercury. The biochemistry of this reaction has been studied and found to involve the transfer of methyl groups from methylcobalamin (methyl- B_{12}) to inorganic mercury (Wood, Kennedy, and Rosen, 1968). Methyl- and dimethylmercury were formed by adding Hg^{+2} to extracts of methanogenic bacteria; the reaction requires ATP and H_2 and forms cobalamin (B_{12}). Methane is a normal product of the reaction, but the presence of mercuric ion leads to the formation of organomercury compounds. The ultimate product is actually dimethylmercury, but increased concentration of Hg^{+2} or acid conditions cause methylmercury to be produced. These workers also demonstrated nonenzymatic methylation using pure methylcobalamin under mildly reducing conditions, and Imura et al. (1971) achieved nearly quantitative methylation in five hours after putting HgCl_2 with methylcobalamin. Another possible mechanism of methylation has been reported by Landner (1971)

in Neurospora crassa. He found that methylation involves the pathway for methionine biosynthesis, resulting in an "incorrect" synthesis of methione; a mercury atom becomes complexed to the homocysteine intermediate, and the methyl group is then transferred to the mercury. Apparently, methylcobalamin is not involved.

The formation of methylmercury in sediments and its occurrence in fish have led to the hypothesis that methylmercury is the only form of mercury involved in transport from sediments to fish (Wood, 1974). Other forms of mercury are thought to be transported by conversion to methylmercury. This theory has several inconsistencies, however, which make it impossible to accept as an adequate explanation of high mercury concentrations in fish. It does not admit the possibility that other forms of mercury, particularly Hg^{+2} and Hg^0 , may be taken up by fish, as has been shown to occur (McKone et al., 1971; Schoper, 1974). There is also some more recent evidence that possibly only 50% or less of the mercury in some fish is in the methyl form (Krenkel, 1973) and that methylmercury in other aquatic organisms is only a small fraction of total mercury (Jernelov and Lann, 1971). Work by Gillespie (1972), who studied mobilization of inorganic and elemental mercury from sediments into guppies, showed that only 30% to 40% of the mercury taken up by the fish was methylated. These findings raise the question of whether other forms of mercury are being taken up or methylmercury is being degraded in the fish. Another weakness is the apparently small amount of methylmercury present in natural sediments. Andren and Harriss (1973) report methylmercury concentrations in sediments from Mobile Bay, the Mississippi River and the Everglades to be about 0.05 ppb, or 0.03% of total mercury. This may be a result of rapid turnover of methylmercury or may indicate that it is not an important form of mercury in these sediments. Another possible explanation may be the presence of bacteria that are able to demethylate methylmercury, producing methane and Hg^0 (Tonomura et al., 1968; Spangler et al., 1973). Billen et al. (1974) were unable to detect any methylmercury in contaminated sediments although methylating bacteria were present. Further work with these sediments revealed the presence of demethylation activity, which was found to greatly decrease added amounts of methylmercury. This is the most serious flaw in the methylmercury hypothesis and some modification seems to be required.

The final path of mercury uptake mentioned in Figure 1 involves complex formation with organic compounds in the sediment, including sulfur-containing proteins and humic materials (Jenne, 1970). This accounts for the strong positive correlation commonly found between mercury concentration and the organic content of sediments (Thomas, 1972, 1973). Reimers and Krenkel (1974) studied mercury uptake by organic compounds containing carboxyl, amine or sulfhydryl functional groups and found a similar capacity for all. At high chloride concentrations (100,000 ppm), only the carboxyl had a decreased amount of uptake. They also found that desorption in water over 24 hours was negligible in all cases. In actual sediments, the form of organic matter most likely to complex with mercury is the humic material, since it is widely distributed and forms the bulk of the organic matter in most sediments. It may be described as amorphous, acidic organic compounds, with molecular weights ranging from hundreds to thousands (Schnitzer and Kahn, 1972). The smaller of these molecules are soluble in both acid and base and are called fulvic acid (FA). Humic acid (HA) molecules are larger and soluble only under

basic conditions. Mercury will react readily with the functional groups of these molecules, particularly with sulfhydryls. Krenkel (1974) summarizes data from several studies showing that organic sediments and humic acid have a high affinity for mercury. More specifically, Strohal and Huljev (1971) were able to show that a stable complex is formed between mercury and humic acid extracted from sediment. Attempts to release mercury from the humic molecule by addition of other cations, including Fe^{+2} , Fe^{+3} , Mn^{+2} , Mg^{+2} , and Zn^{+2} , were unsuccessful. A small amount of mercury was released by the addition of 0.65% NaCl. DeGroot et al. (1971) report a considerable loss of mercury (about 90%) from sediments as they are transported into estuaries and salinities of 1.6% Cl. In freshwaters, mercury may be resolubilized as an organic complex and released to the water column in that form, but this process is probably not significant (Fagerström and Jernelöv, 1972; Lindberg and Harriss, 1974). One other mechanism of release is possible for mercury associated with humic acid, i.e. reduction of the mercury by the humic acid to the elemental form, Hg^0 , which is both volatile and slightly soluble. Szilagyi (1971) reported that Fe^{+3} can be reduced to Fe^{+2} by humic acid in solution, and Szalay and Szilagyi (1967) report a similar process for vanadium. Reduction of mercury and formation of Hg^0 in this way can lead to the release of mercury from sediments.

CADMIUM AND CHROMIUM

Although the behavior of cadmium and chromium does not appear to be as complex as mercury, their presence in natural waters is significant because of their widespread industrial uses, possible frequent occurrences in waste stream effluents and their toxicity (Cheremisinoff and Habib, 1972). Gardiner (1974a, b) investigated the extent of formation of labile complexes of cadmium and noted that a substantial proportion of the total cadmium in river and lake water will be present as the free cadmium ion. Gardiner also pointed out that cadmium concentrations depended upon the pH of the system and the ligand availability. However, humic acids were discovered to be the main component responsible for cadmium adsorption.

Little is known about the behavior of chromium in natural waters, but its toxicity is a function of the temperature, pH and the valence form (Cr^{+3} and Cr^{+6}) (Cheremisinoff and Habib, 1972). However, little is known about the affinity of chromium towards natural humic and fulvic acids and the role of these natural organic materials in its removal from water.

SECTION II

CONCLUSIONS

Except when contamination is very high, mercury uptake by sediment is nearly total, resulting in extremely low concentrations of dissolved mercury in natural waters. Mercury is associated to a large extent with the organic fraction of the sediments. Uptake is influenced by the pH and Eh, probably by causing changes in the molecular configuration and the functional groups of the humic and fulvic molecules.

Purified humic acid from several different sediments is able to reduce mercuric chloride in solution to elemental mercury. The reaction is inversely proportional to pH and totally inhibited by sulfide. When the ratio of humic acid to mercury is less than 1, release is slow. The rate increases as the proportion of humic acid increases, until about 20% of added mercury is reduced in 1 week at ratios of 10 to 100. As the amount of humic material is further increased, the reaction slows until there is almost total inhibition at a ratio of 1000. The same process occurs using whole sediments in place of purified humic acid, but the amounts of release are much lower. Comparison of release with uptake patterns leads to the hypothesis that mercury associated with larger humic molecules will be complexed and that any excess over this amount will be reduced by smaller fulvic acid molecules.

Most mercury added to fresh waters will be inorganically complexed and removed from the active mercury cycle. A small amount will be available for methylation. A larger fraction will be organically complexed and potentially able to be cycled as Hg^0 . Release of elemental mercury from lake sediments will depend on both the amount and form of organic material present and on the Eh and pH. Even though release of both elemental and methylmercury is very slow, high concentrations may be obtained in the biota. Methylmercury tends to accumulate in fish, while Hg^0 is continually recycled between sedimentary organics and fish.

Cadmium also shows a high affinity for natural organic materials (humic components) in reducing environment; chromium appears to have less affinity for the natural organic component. Cadmium and chromium do not participate in transformations that are equivalent to the mercury reduction pathways in nature.

SECTION III

METHODS

SEDIMENT UPTAKE AND RELEASE

Sediments used in the laboratory to study mercury uptake and release were collected by Ekman dredge from several freshwater environments. Most of the work was done with sediment from Lago Pond, a small farm pond in Winterville, Clarke County, Georgia. Also used were sediments from Par Pond, a nuclear reactor cooling pond on the Savannah River Plant, and the Clark Hill and Hartwell reservoirs on the Savannah River. These sediments are described in Table 1. The Lago Pond sediments were very finely divided, as indicated by the high moisture content, and contained a normal amount of mercury. There was more mercury in the sediment of Par Pond, which is known to be contaminated. The other two sediments were chosen to provide a contrast, since they were very sandy, and the mercury concentration was low. The mercury used was of two types, reagent grade mercuric chloride (HgCl_2) and radioactive mercury-203, supplied by ICN as mercuric nitrate ($\text{Hg}(\text{NO}_3)_2$) in 1 N HNO_3 . The ^{203}Hg was used in small amounts as a tracer, and a mixture of these two compounds resulted in a uniformly labeled pool of mercury by the processes of ionization and exchange of mercuric ion for undissociated mercury atoms. This method was used by Clarkson and Greenwood (1968) and produced results consistent with the assumption of uniform labeling. The process is reportedly rapid, and, in the work reported here, at least 15 minutes elapsed between the time of labeling and initiation of an experiment. Counting was done with an ORTEC well-type scintillation counter with a 2 x 2 inch (5.08 cm) NaI crystal. Prior to beginning an experiment, the specific activity of the mercury in the reaction mixture was calculated by counting a 3-ml aliquot and dividing by the amount of mercury present. Subsequent experimental counts were also made of a 3-ml aliquot, and the amount of mercury present was calculated by dividing the observed cpm, after correction for background and decay, by the specific activity calculated initially. To facilitate comparison, the results are presented as a percentage of the initial amount of added mercury.

Other routine conditions and procedures included cleaning all glassware with concentrated nitric acid and doing all experiments at room temperature, 22° to 25°C. Eh was determined by inserting an Ag/AgCl electrode and a shiny platinum electrode into the sediments and measuring the potential generated between them on a voltmeter. The pH was controlled in the range of 5.9 to 8.2 by the use of a borate buffer made to the desired pH by adding solid boric acid to a saturated solution of sodium borate. Lower pH resulted when distilled water was used in place of the buffer because of the added HgCl_2 and HNO_3 (the ^{203}Hg solvent). In all experiments there was no change in pH from

the beginning to the end.

Studies of the uptake of mercury were done by mixing sediment, distilled water or buffer, labeled mercury in a stoppered flask, sealing under nitrogen, and shaking. The specific activity of the mercury added was determined before addition of the sediments to avoid problems with taking a homogeneous aliquot. After shaking, the mixture was centrifuged for 20 minutes at 14,000 rpm and

TABLE 1. SEDIMENT COMPOSITION

Sediment	Dry wt (%)	Ash wt (%)	Total Hg (ppm-dry wt)	Hg in KOH extract (ppm-dry wt)
Lago Pond	18.6	15.7	0.15	N.D.*
Par Pond	22.2	19.9	0.39	0.11
Clark Hill	54.6	50.7	0.11	N.D.
Lake Hartwell	43.6	40.5	0.09	N.D.

*Not determined.

the mercury in the supernatant was counted. The solid sediments were further analyzed by extraction with a fivefold excess (w/v) of 0.1N KOH under N₂ overnight, a standard procedure to solubilize total humic and fulvic acid (Schnitzer and Khan, 1972). After a 20 minute centrifugation at 14,000 rpm, the KOH supernatant was counted and then acidified to pH 1 with concentrated HCl to precipitate the humic acid. The fulvic acid stays in solution and, after further centrifugation at 14,000 rpm, this supernatant was counted. In all steps, the amount of mercury in the solid fraction was estimated by difference (See Figure 2).

Release of mercury from sediments was studied using the apparatus described in Figure 3. Two types of experiments were done, one using purified humic acid and the other using whole sediment as the source of humic acid. Pure humic acid was extracted from sediment with KOH, as described above (Fig. 2). Further cleanup was done by shaking the humic precipitate with several changes of 1% HF-HCl, then redissolving it in 0.1N KOH and dialyzing against distilled water until the pH was near neutral, followed by freeze-drying (Table 2). For use in the reaction flask, the humic acid was dissolved in 0.1N KOH and added in that form, along with the labeled mercury and distilled water or buffer to a volume of about 50 ml. In experiments using whole sediments, no buffer was used and the water (30 ml) and mercury

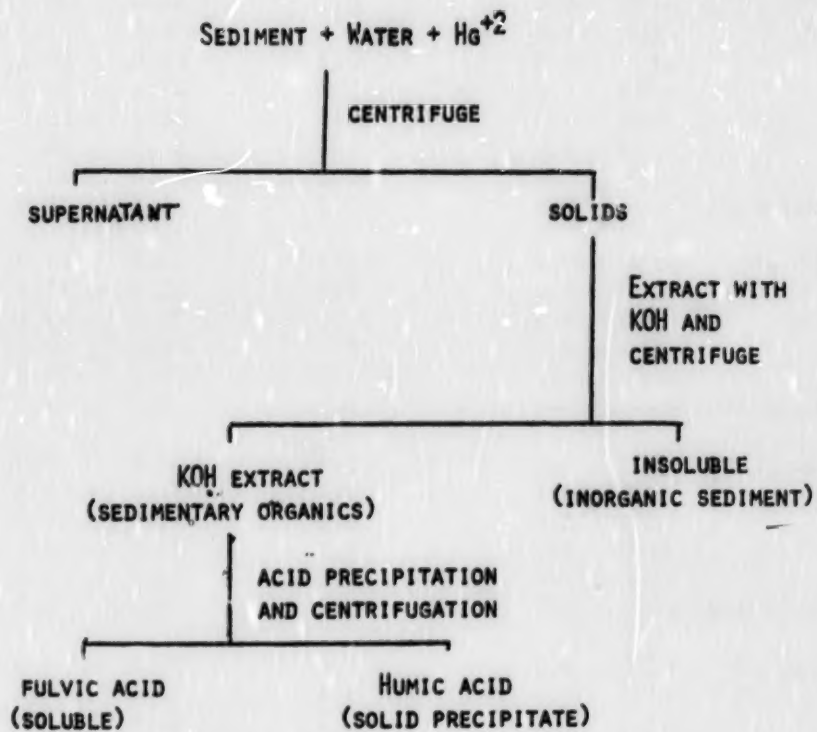


Figure 2. Extraction Process

TABLE 2. UPTAKE OF 10 MICROGRAMS ADDED MERCURY BY 10 GRAMS (WET WT)
LAGO POND SEDIMENT.

Treatment	Eh (mv)	pH	% of added Hg in sediment fractions			
			Interstitial Water	KOH Extract	FA	HA ^a
Low Eh, low pH	-100	4.7	0.8	40.2	21.0	19.2
Do.	-100	4.7	0.1	50.3	21.5	28.8
Do.	-100	4.8	0.1	51.8	23.7	28.1
Do.	-100	4.8	0	54.8	23.4	31.4
Do.	-150	4.8	0.3	47.6	20.6	27.0
Do.	-150	4.8	0.2	47.1	20.4	26.7
Do.	-150	4.9	0.2	44.7	19.7	25.0
Do.	-150	4.9	0.1	57.6	20.5	27.1
Mean	----	---	0.2	48.0	21.3	26.7
Standard Deviation	----	---	0.3	4.5	1.5	3.5
Low Eh, high pH	-150	8.0	1.6	23.7	13.8	9.9
Do.	-150	8.0	3.0	32.7	18.6	14.1
Do.	-150	8.0	4.6	23.6	13.2	10.4
Do.	-150	8.0	2.5	35.9	20.1	15.8
Mean	----	---	2.9	29.0	16.4	12.6
Standard Deviation	----	---	1.3	6.3	3.4	2.9
High Eh, low pH	+220	3.1	0.3	36.1	5.0	31.1
Do.	+220	3.1	0.1	32.6	5.1	27.5
Do.	+220	3.1	0.2	31.9	4.4	27.5
Do.	+220	3.1	0.1	31.3	4.1	27.2
Mean	----	---	0.2	33.0	4.7	28.3
Standard Deviation	----	---	0.1	2.2	0.5	1.9
High Eh, high pH	+220	8.0	5.0	25.2	3.5	21.7
Do.	+220	8.0	5.1	25.6	3.5	22.1
Do.	+220	8.0	5.1	25.4	3.6	21.8
Do.	+220	8.0	5.1	24.7	3.6	21.1
Mean	----	---	5.1	25.2	3.6	21.7
Standard Deviation	----	---	0.1	0.4	0.1	0.4
Intermediate pH	-150	5.9	0.9	25.9	13.6	12.3
Do.	-150	6.0	0.8	25.9	13.7	12.2
Do.	-150	7.2	2.8	22.1	10.9	11.2
Do.	-150	7.1	1.8	21.0	11.0	10.0
Do.	+ 50	5.7	0.2	23.9	3.5	20.4
Do.	+ 50	5.7	0.1	23.2	3.1	20.1
Do.	+ 50	7.2	1.1	21.0	3.8	17.2
Do.	+ 50	7.2	1.1	20.0	3.6	16.4

^aDifference between KOH Extract and FA

were mixed in the flask and counted to determine the specific activity before the sediment was added. Bubbling with N_2 began immediately in experiments with pure humic acid, but was delayed 48 hours in sediment experiments to allow the mercury to reach equilibrium between water and sediments. At intervals during the experiment, the $KMnO_4$ trap was removed and replaced with a fresh one, with only a short break in the gas flow. The mercury present in the trap was quantified by clearing the $KMnO_4$ and any precipitate with 25% hydroxylamine hydrochloride and counting a 3 ml aliquot. At the end of an experiment, about 80-90% of the mercury initially added could be recovered, the losses most likely to the glassware and tubing. To verify that the volatile mercury species was actually Hg^0 , a liquid trap containing 1% cysteine in tris buffer was placed in the apparatus just before the permanganate trap. The cysteine will complex with charged mercurials such as Hg^{+2} and CH_3-Hg^+ (Clarkson and Greenwood, 1970) and retain them in solution. There was no such retention of the volatile mercury species under normal experimental conditions. More positive confirmation was obtained by passing the effluent gas from the reaction flask through the absorption cell of an atomic absorption spectrophotometer set to determine elemental mercury; a strong response verified its presence.

Analysis of natural mercury concentrations in whole sediments and in KOH extracts was done by a modification of the reduction-aeration technique of Hatch and Ott (1968). Samples of whole sediment (5 g or less) or extracts were wet digested and oxidized at room temperature overnight by the addition of 10 ml each of concentrated HNO_3 and H_2SO_4 , about 200 mg potassium persulfate and excess 5% (w/v) $KMnO_4$. After this was complete, any excess $KMnO_4$ was reduced with hydroxylamine hydrochloride and all samples made to an equal volume. The sample was then totally reduced with excess stannous chloride and aerated into a closed system, passing through a 10 cm absorption cell in the light path of a Perkin-Elmer Model 303 atomic absorption spectrophotometer set to detect mercury. The absolute amount of mercury present was determined from a standard curve prepared in the same way and the results converted to micrograms of mercury per gram dry weight of the sediment (ppm-dry wt).

UPTAKE AND RELEASE BY FISH

For this study the small mosquito fish, Gambusia affinis, was found to be a particularly good experimental organism because of its small size 2.5 to 4 cm. and its hardy tolerance to experimental handling. The volatility of elemental Hg necessitated the use of closed glass vessels in the uptake experiments. Figure 3 shows the design of the apparatus. A small droplet of metallic mercury was placed in a glass cup and suspended above 1.5 liters of aged tap water until a satisfactory concentration was reached (12 to 48 hours) at ambient temperature conditions ($\sim 23^\circ C$). Preliminary investigation indicated this approach of solubilizing metallic mercury was more advantageous than merely placing droplets of the metal into the water (Holm, personal communication). The concentration of mercury in the water was measured by withdrawing several ml. of water from the side port, and determining total mercury spectrophotometrically with a Perkin Elmer Atomic Absorption Model 303. The mercury droplet was then removed and the fish introduced through the side port, below the water surface. The water in the test flasks was not stirred prior to the introduction of the fish. The fish density in the test flasks

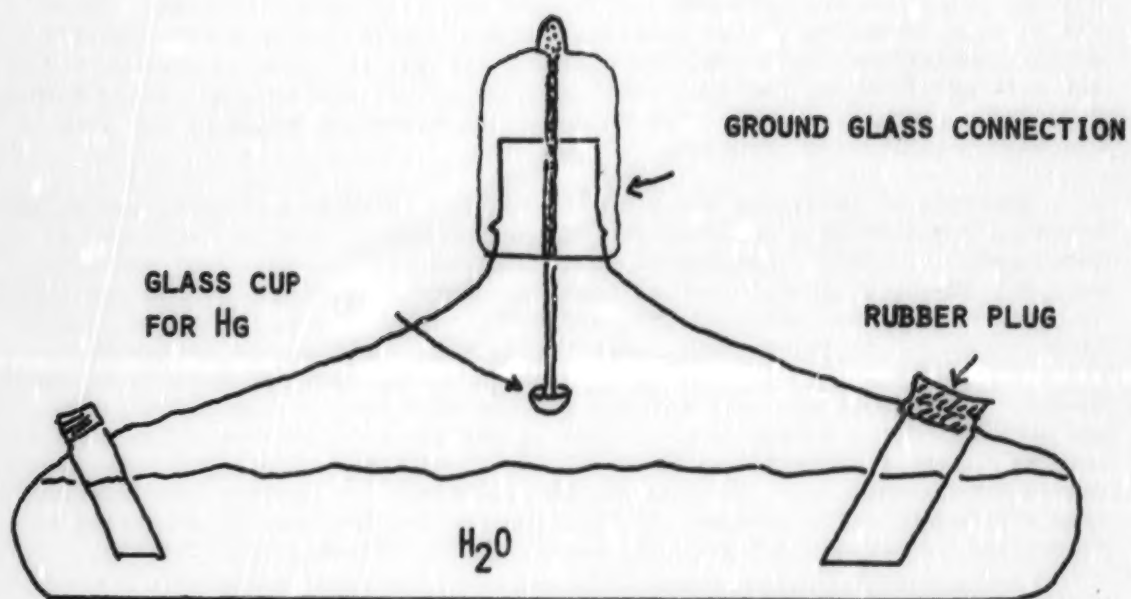


Figure 3. Experimental apparatus for Hg⁰ accumulation by fish

was 1 g. weight of fish per 1 liter water, which was approximately 4 fish per flask (American Public Health Association, 1971). After varying time periods, all the fish in a test flask were removed and analyzed individually for total mercury by the flameless mercury techniques, as outlined by Uthe et al. (1970) and Kopp et al. (1972). It was found that in this analytical procedure, the acid digestion step should proceed for 2 to 4 hours, until the digestate is clear, to ensure an accurate total mercury determination. Direct uptake of elemental mercury by fish was measured as the increase in body concentration of mercury with time in fish exposed to Hg^0 -contaminated water as compared to control fish held in Hg^0 -free water.

The water in the test flasks in the Hg^0 uptake experiments ranged from 80 to 170 ppb (= parts per billion, $\mu\text{g}/\text{l}$) total mercury. The range in concentration is due to the varying size of the mercury droplet placed in the cup. These concentrations are high as compared to the solubility of metallic mercury in water devoid of air and under a vacuum at 30°C , which is only 25 ppb (Stock et al., 1934). The increased solubility of metallic mercury can be explained by oxygenation of the water and the presence of Hg-binding organic impurities in tap water (Stock et al., 1934). The actual ratio of Hg^0 to total Hg in the test flasks was measured by analyzing aliquots of the test solutions without the addition of reagents as compared with the mercury concentration of a similar sized aliquot to which all reagents had been added, according to the Kopp et al. (1972) procedure. The ratio of Hg^0 to total mercury ranged from 78-89% (ave. 84%) before the addition of fish. The binding of organics, present in tap water, to Hg^0 could account for part of the differences observed between Hg^0 and total mercury. Mercury readily forms stable organic complexes which do not respond to the flameless technique without the addition of reagents (Kopp et al., 1972). Volatility of Hg^0 from the sample aliquot could also account for the difference between Hg^0 and total mercury concentration. After the addition of fish, the ratio of Hg^0 to total did not significantly change indicating no preferential uptake of one form over the other.

The extent of body surface adsorption of elemental mercury, as opposed to actual tissue incorporation, was measured as the difference between the total mercury concentration of Gambusia which had been surface scraped and rinsed prior to digestion and unscraped, digested Gambusia. Gill tissue was not removed.

Direct uptake experiments were of two types: 1) continuous, where fish were continuously exposed to known mercury concentrations for known time periods and 2) periodic exposures, where fish were exposed to known mercury concentrations for 10 two-hour periods (1 period/day) and held in mercury-free water between exposure periods. The first types of experiments were short term with a maximum exposure duration of 24 hours. The second type permitted longer experimental durations since it was not necessary to provide supplemental oxygenation or remove water from the exposure vessels, but the total cumulative exposure of the fish to mercury was just 20 hours. The uptake rate of Hg^0 was compared to the uptake of ionic mercury, by conducting similar uptake experiments using HgCl_2 .

Elimination rates were determined by placing fish of known Hg body burdens into open vessels of mercury-free water and measuring changes in body levels of total mercury with time. Preliminary elimination tests were made with ^{203}Hg . Concentrations of mercury in the residual water were not measured.

Biotransformation of inorganic mercury by fish was investigated by in vitro and in vivo methods. In vitro methylation was attempted first to see if the potential for methylation by fish livers existed. These methylation experiments were conducted using procedures similar to those outlined by Imura et al. (1972). Livers from two species of fish were used, bluegills (Lepomis macrochirus) and rainbow trout (Salmo gairdneri). Since some field data (unpublished as yet) have suggested that population crowding in fish may be a factor in the extent to which methylation occurs, livers from both normal-size populations as well as overstocked populations were used. The livers were homogenized with a few ml. of ice-cold deionized water, in a tissue grinder to which a very small quantity of clean sand was added. To these homogenates, 10 ppm of HgCl_2 were added and the homogenates plus control homogenates were incubated for at least 5 hours in the dark in a $32\text{--}33^\circ\text{C}$ water bath. After incubation, the homogenates were analyzed for methylmercury. Several replications of the above experiment were made with such modifications as the addition of a 6.8 pH H_2PO_4 buffer, performing preliminary experimental steps under light and dark conditions (since B_{12} is light sensitive), allowing various incubation times, using fresh and frozen livers, and using two different extraction procedures for the methylmercury determination. Quantification of the methylmercury concentration was made on a Perkin Elmer Gas Chromatograph using a KBr saturated SE-30 glass column and an electron capture detector. The operating conditions were similar to those in the Environmental Protection Agency (EPA) procedure published by Longbottom et al. (1972).

Liver tissue was particularly difficult to extract because of its high fatty constituency, which has a tendency to form unusually thick, insoluble emulsions. The Longbottom et al. (1972) procedure, while quite satisfactory for muscle tissue, was unsatisfactory for the analysis of liver tissue. Better results were obtained using Westö's (1968) procedure, with a few modifications. However, completely clean separations were not achieved even with these modifications of the Westö procedure. This is very significant when trying to detect small percentages of methylmercury formation. The use of glass wood rinsed with solvent has been found to be effective in breaking up loose emulsions, but was of no use in these very thick, stable emulsions.

In vivo methylation experiments were also conducted since the lack of positive results on the in vitro methylation experiments did not preclude the possibility that such formation may occur in vivo. Gambusia were periodically exposed to both Hg^0 and HgCl_2 (comparable concentrations and conditions) for a maximum of 10 two-hour periods and held in mercury-free water for additional periods up to 38 days. At designated times, or upon death, fish were removed and the whole body extracted according to the Longbottom procedure. The methylmercury concentrations of the test fish were then compared to those of extracted controls. In these extractions the possibility of emulsion formation

was greatly reduced or completely eliminated by the manner in which the extraction flasks were shaken, i.e. with a moderately slow circular motion with the stoppered end of the flask positioned downward.

SECTION IV

RESULTS

SEDIMENTS

For the purpose of studying mercury uptake and distribution in the interstitial and organic fractions of the sediment, 10 μ g of labeled mercury were added to approximately 10 g of wet Lago Pond sediment to produce a concentration of about 5.5 ppm (dry wt basis) or almost 40 times the natural amount. This simulates the amount of mercury in a moderately to heavily polluted system (Krenkel, 1973). The pH was adjusted by adding 30 ml of buffer or distilled water to the reaction mixture, those above pH 5 requiring buffer. The Eh of the sediment was naturally -100 to -150 mv; sediment of higher Eh was prepared to aeration. The contents of the reaction flask were sealed under nitrogen during the initial shaking period and during the KOH extraction to maintain consistency of results. A series of preliminary experiments was done to determine the time necessary for the mercury concentration to reach equilibrium between the water and the sediment. With moderate (100rpm) shaking, this occurred within 2 hours, and no difference was observed with up to 72 hours of shaking. Therefore, the uptake experiments were done with a minimum of 2 hours of shaking.

The results presented in Table 2 are divided into four groups based on Eh and pH, with a fifth group of intermediate results. The percentages of the mercury initially added to the system which were found in the various fractions are given in the last four columns. The amount in the interstitial water is essentially that which is in equilibrium with the sediment. The KOH extract represents total extractable organics and is the sum of the fulvic and humic acids. The percentage of added mercury bound to the inorganic fraction of the sediment is not included in the table but may be calculated by subtracting the combined percentages in the interstitial water and the KOH extract from 100%. The inorganic fraction was not further fractionated to determine specific binding sites of the mercury. These are assumed to be primarily sulfides and adsorbed forms.

The mean and standard deviations of the data in the different Eh-pH groups are also given in Table 3 to facilitate comparisons between the amounts of mercury taken up under the different conditions. Overall, many of the differences between means do not appear to be very great, although most of them are statistically significant. In the interstitial water, the important point to note is the very small amount of mercury usually present in this fraction. Only under conditions of high pH does it rise above 1%, and this is something of an artifact, since organic material containing complexed mercury material begins to become soluble at pH 7, and the water did in fact become slightly

TABLE 3. UPTAKE OF MERCURY BY LAGO POND AND CLARK HILL SEDIMENTS

Source of Sediment and amount of mercury added (mg)	% of added Hg in sediment fractions			
	Interstitial Water	KOH Extract	FA	HA*
Lago Pond ⁺ :				
1	0.1	18.9	0.1	18.8
2	0.1	16.3	0.1	16.2
5	0.1	14.1	0.1	14.0
10	0.9	11.3	1.6	9.7
50	42.4	5.4	3.6	1.8
100	64.9	3.9	2.8	1.1
500	84.1	2.3	1.9	0.4
1000	88.5	1.4	1.3	0.1
Clark Hill:				
0.01	0.1	29.5	1.8	27.7
0.1	0.1	35.6	1.4	34.2
0.5	0.4	35.8	9.6	26.2
1	1.6	26.5	10.8	15.7
2	3.6	25.3	15.5	9.8
5	11.2	21.1	16.5	4.6
10	20.7	17.1	12.9	4.2
50	68.6	7.7	6.0	1.7
100	82.5	4.8	3.7	1.1
500	94.0	2.0	1.6	0.4
1000	99.1	1.3	1.1	0.2

* Difference between KOH Extract and FA.

+ Average of two determinations.

colored. The amount of soluble mercury not complexed with organics is probably best indicated by the results under low pH. Oxidation-reduction potential has no apparent effect on interstitial mercury concentrations, so uptake of mercury is essentially total under normal conditions of these sediments. This is in agreement with the results of Lindberg and Harriss (1974), who found average interstitial mercury concentrations of about 0.1% of total mercury in several different sediments.

The amount of mercury present in the humic acid fraction increased somewhat with Eh but was negatively correlated with pH. Significant correlations between pH and HA uptake were calculated separately under conditions of both positive and negative Eh ($r = -0.79$, 10 df and $r = -0.84$, 14 df, respectively). The amount of mercury associated with humic acid at the low pH usually found in sediments is between 20-30% of the total mercury present. The other part of the organic fraction of the sediments, the fulvic acids, was strongly influenced by Eh in its ability to take up mercury, percentages being several times higher at low Eh. The pH was also an influence, being negatively correlated with mercury uptake, as in the case of humic acid ($r = -0.78$, 10 df at high Eh; $r = 0.65$, 14 df at low Eh). The negative correlation between organic uptake and pH was also noted by Reimers and Krenkel (1974), who found decreasing uptake by soluble organics as pH increased.

A second set of experiments was done, using increasingly higher amounts of added mercury, for the purpose of determining the capacity of these sediments to take up mercury and to relate this uptake to the mercury release described later. From 1 to 1000 mg of labeled inorganic mercury were added to samples of sediment from Lago Pond (10 g) and Clark Hill (3 g), producing extremely high mercury concentrations. No buffer was used, so pH remained low, and all experiments were done under N_2 and at low Eh. Instead of shaking, all of the mixtures were allowed to sit for 48 hours, during which time the mercury concentrations in the water (30 ml) and sediment reached equilibrium. The standard extraction procedure was followed (Fig. 2). Table 3 presents the percentages of total mercury present in the various fractions. The most striking aspect of these data is the amount of mercury found in the interstitial water as the total amount is increased. In both sediments there was a sudden increase in interstitial concentrations when additions exceeded 10 mg although the increase was somewhat more gradual in Clark Hill sediment. Apparently the capacity of these sediments to take up mercury had been exceeded. The percentage of mercury in the KOH extract and in the humic acid fraction continued to decline slowly, showing a noticeable decline when additions exceeded 10 mg. The percentage uptake by fulvic acid was unusual--it increased in the middle range of added mercury and declined at either extreme.

The association of mercury with sedimentary organic materials, shown above, and the possibility of further reaction by mercury reduction, mentioned in the introduction, led to the following experiments on mercury release. Labeled inorganic mercury and purified humic acid, extracted from either Lago Pond or Par Pond sediments, were mixed in the reaction flask (Fig. 4) with 50 ml buffer at the indicated pH. When nitrogen was bubbled through the solution, Hg^0 vapor was released, trapped and quantified. The formation and release of elemental mercury was verified as previously mentioned, indicating that mercury

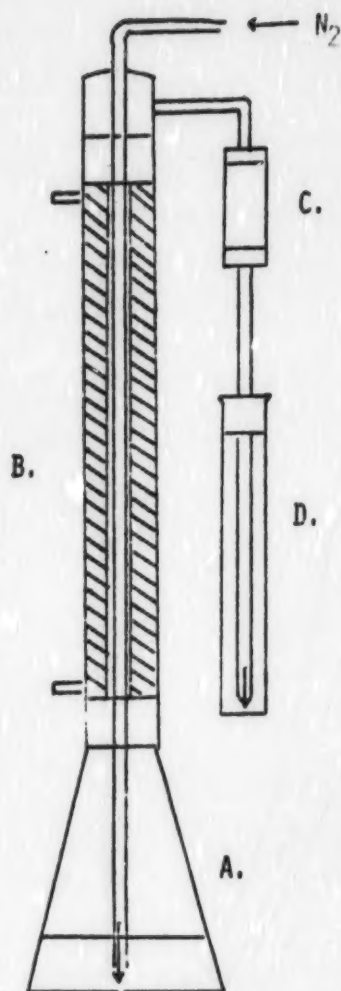


Figure 4. Experimental apparatus for elemental mercury volatilization

- A. Reaction flask
- B. Water-filled condenser
- C. Anhydrous trap
- D. Mercury trap, containing 20 ml 5% (w/v) potassium permanganate and 5 ml 18N. sulfuric acid.

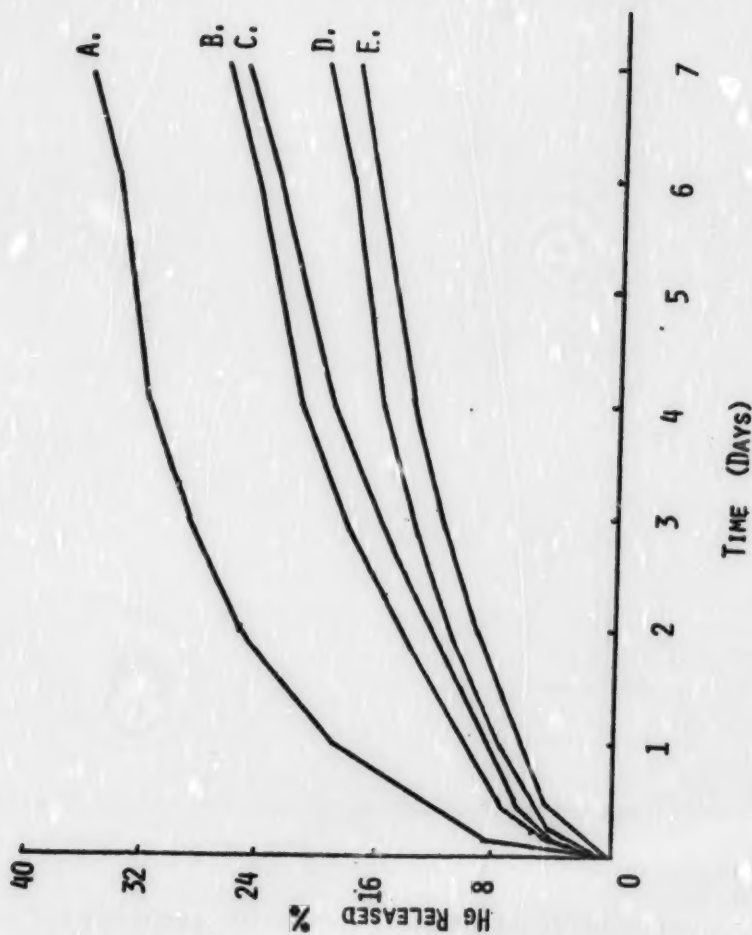


Figure 5. Release of Hg^0 in the presence of purified humic acid at different pH's. Each reaction flask initially contained 200 μg Hg and 1 mg HA.

- A. pH 4.1 Lago Pond HA
- B. pH 6.5 Par Pond HA
- C. pH 7.5 Par Pond HA
- D. pH 7.3 Lago Pond HA (avg. of two separate reactions)
- E. pH 8.2 Par Pond HA

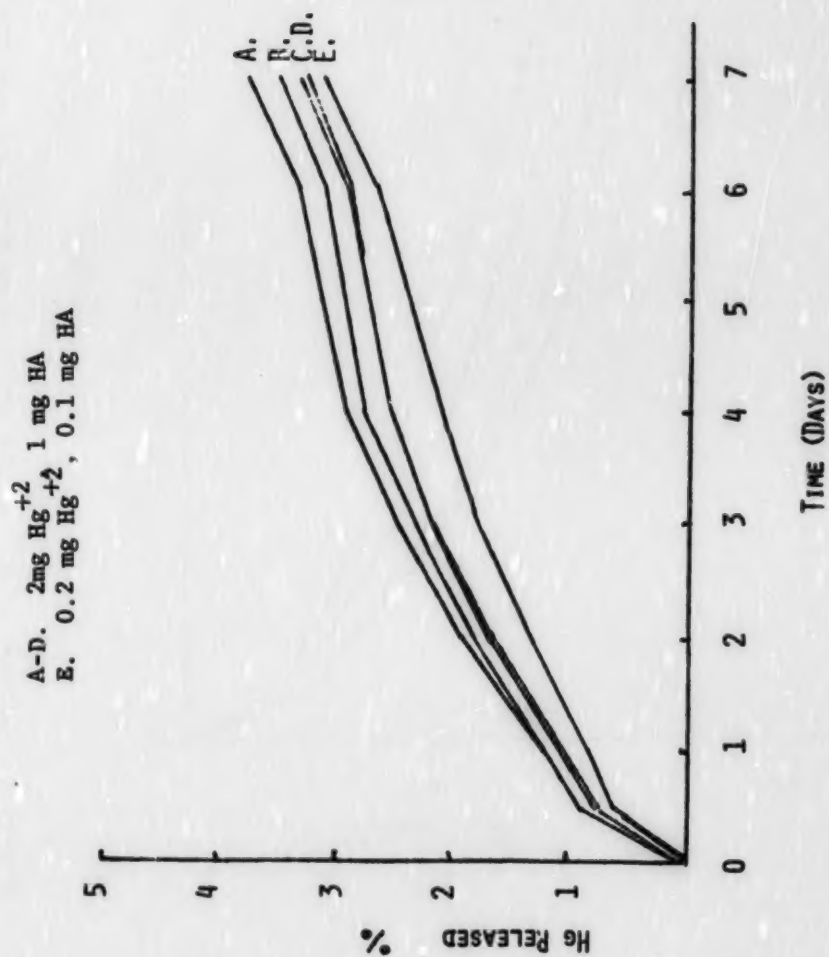


Figure 6. Release of Hg⁰ in the presence of purified Lago Pond humic acid; HA:Hg = 0.5

A-C. HA:Hg = 5, 0.2 mg Hg^{+2} , 1 mg HA
 D. HA:Hg = 4, 25 μg Hg^{+2} , 100 μg HA

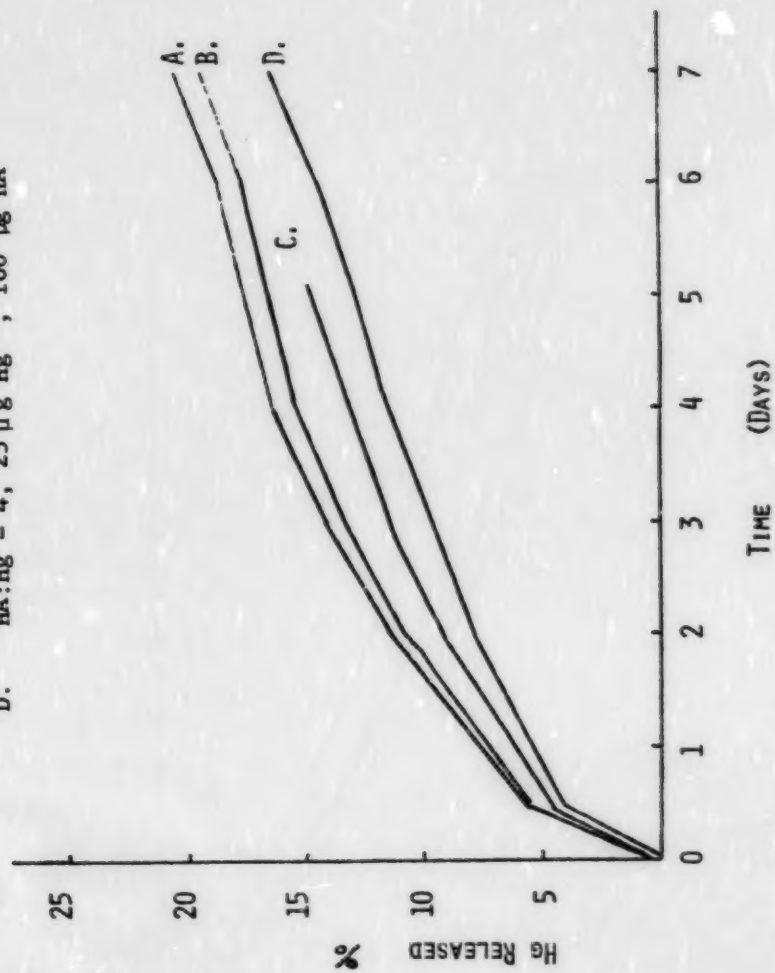


Figure 7. Release of Hg^0 in the presence of purified Lago Pond humic acid.

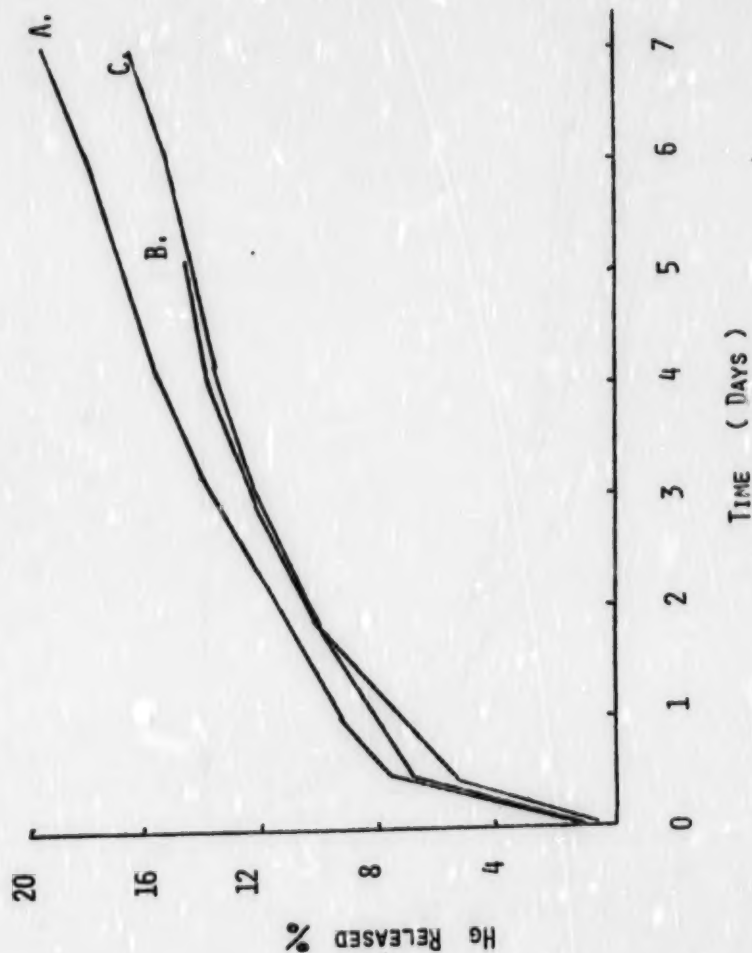


Figure 8. Release of Hg^0 in the presence of purified Lago Pond humic acid
 A. HA:Hg = 50, 0.2 mg Hg_2^{+2} , 10 mg HA
 B. HA:Hg = 43, 23 μg Hg_2^{+2} , 1 mg HA
 C. HA:Hg = 40, 25 μg Hg_2^{+2} , 1 mg HA

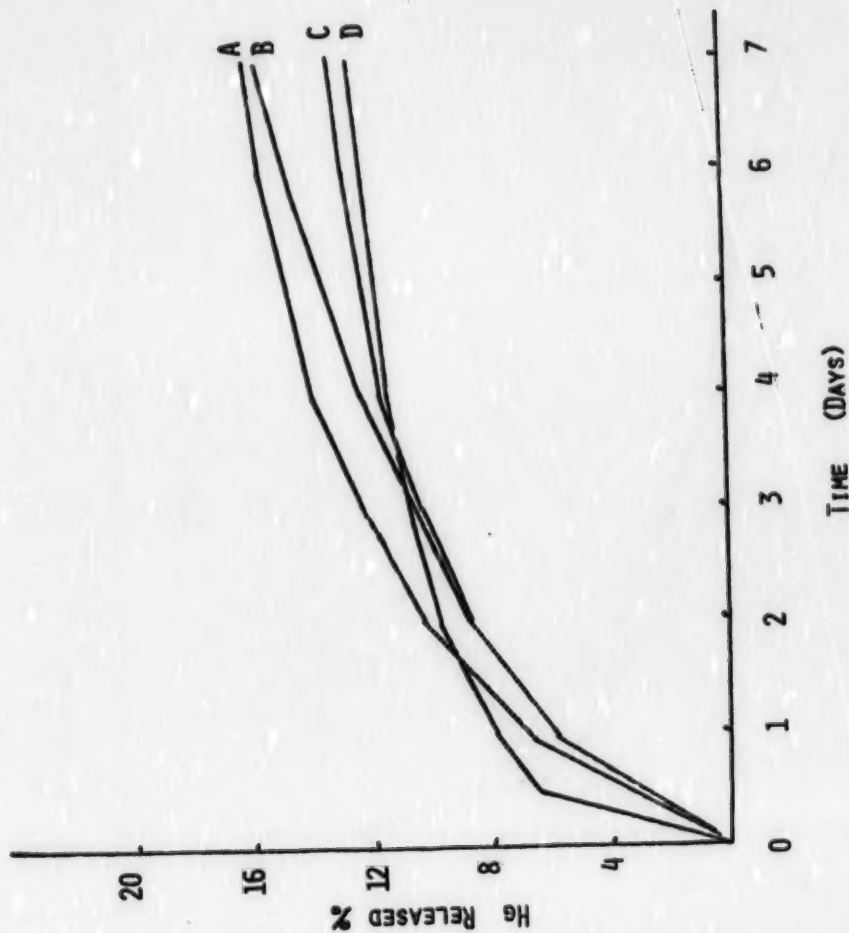


Figure 9. Release of Hg^0 in the presence of purified Lago Pond humic acid.
 A-C. HA:Hg = 100, 10 μg Hg_{+2} , 1 mg HA
 D. HA:Hg = 400, 25 μg Hg_{+2} , 10 mg HA

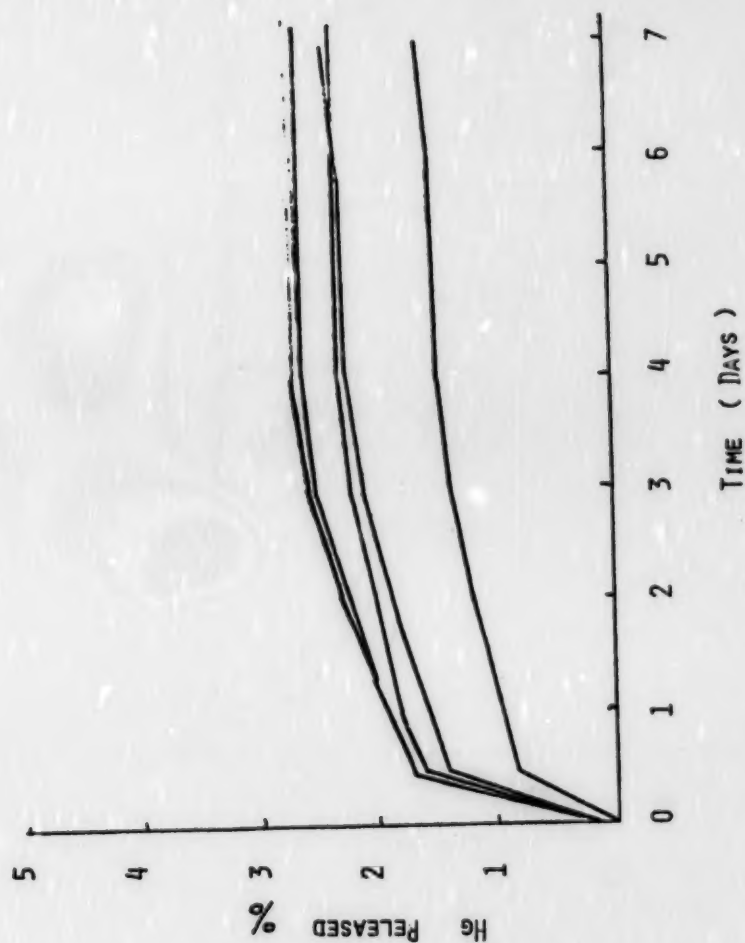


Figure 10. Release of Hg^{2+} in the presence of purified Lago Pond humic acid. HA:Hg = 1000, 10 micrograms Hg^{2+} , 10 mg HA (Results of 5 individual determinations)

- A. HA:Hg = 5 (n = 3)
 B. HA:Hg = 100 (n = 3)
 C. HA:Hg = 0.5 (n = 5)
 D. HA:Hg = 1000 (n = 5)

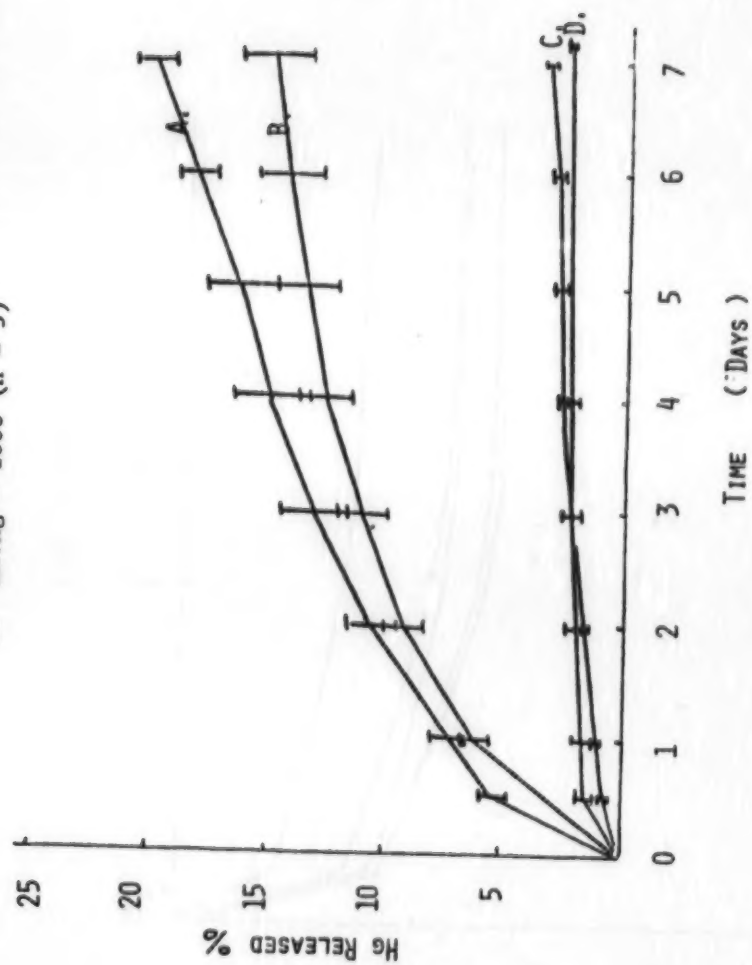


Figure 11. Summary of the release of Hg^0 in the presence of purified Lago Pond humic acid. Error is ± 1 standard deviation

- A. Initially 0.2 mg Hg^{+2} , 1 mg HA, add 1 mg HA
 B. Initially 23 μg Hg^{+2} , 1 mg HA, add 1 mg HA
 C. Initially 25 μg Hg^{+2} , 10 mg HA, add 10 mg HA
 D. Initially 0.2 mg Hg^{+2} , 0.1 mg HA, add 0.1 mg HA
 Arrow indicates time of addition

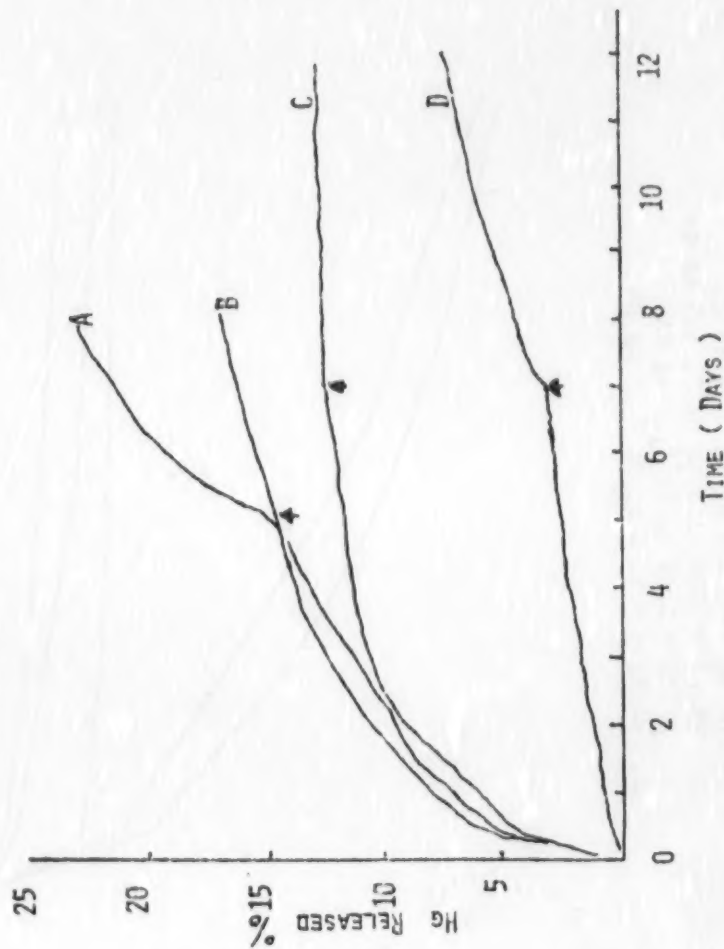


Figure 12. Effect of an increase of humic acid on the release of Hg^{+2}

- A. HA: Hg = 10, 0.1 mg Hg^{+2}
 B. HA: Hg = 5, 0.2 mg Hg^{+2} , 1 mg HA
 C. HA: Hg = 1000, 1.5 mg Hg^{+2} , 1.5 mg HA
 D. HA: Hg = 1, 1 mg Hg^{+2} , 1 mg HA
 E. HA: Hg = 0.5, 2 mg Hg^{+2} , 1 mg HA

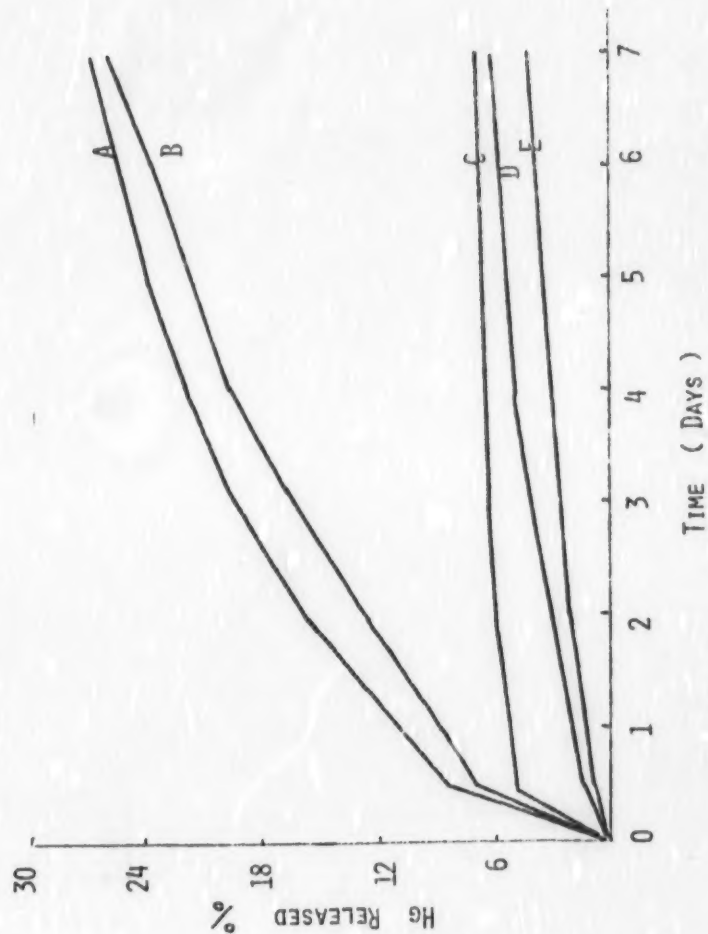


Figure 13. Release of Hg^0 in the presence of purified Par Pond humic acid

- A. HA:Hg = 10, 0.1 mg Hg⁺², 1 mg HA
 B. HA:Hg = 1, 1 mg Hg⁺², 1 mg HA
 C. HA:Hg = 1000, 1.5 μ g Hg⁺², 1.5 mg HA
 D. HA:Hg = 0.5, 2 mg Hg⁺², 1 mg HA

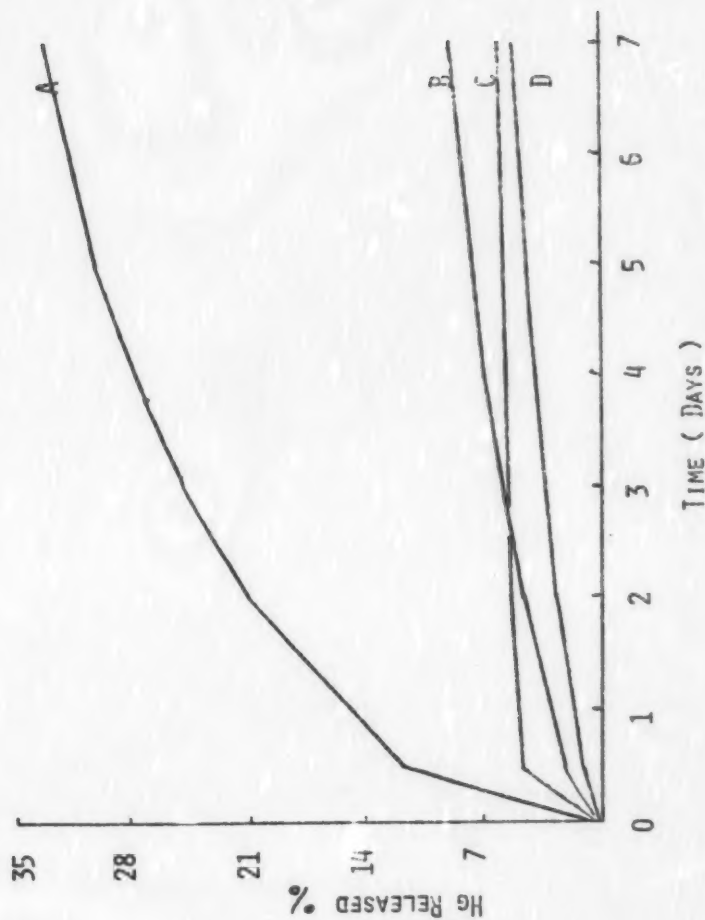


Figure 14. Release of Hg⁰ in the presence of purified Lake Hartwell humic acid

- A. HA:Hg = 10, 0.1 mg Hg⁺², 1 mg HA
 B. HA:Hg = 1000, 1 microgram Hg⁺², 1 mg HA
 C. HA:Hg = 1, 1 mg Hg⁺², 1 mg HA
 D. HA:Hg = 0.5, 2 mg Hg⁺², 1 mg HA

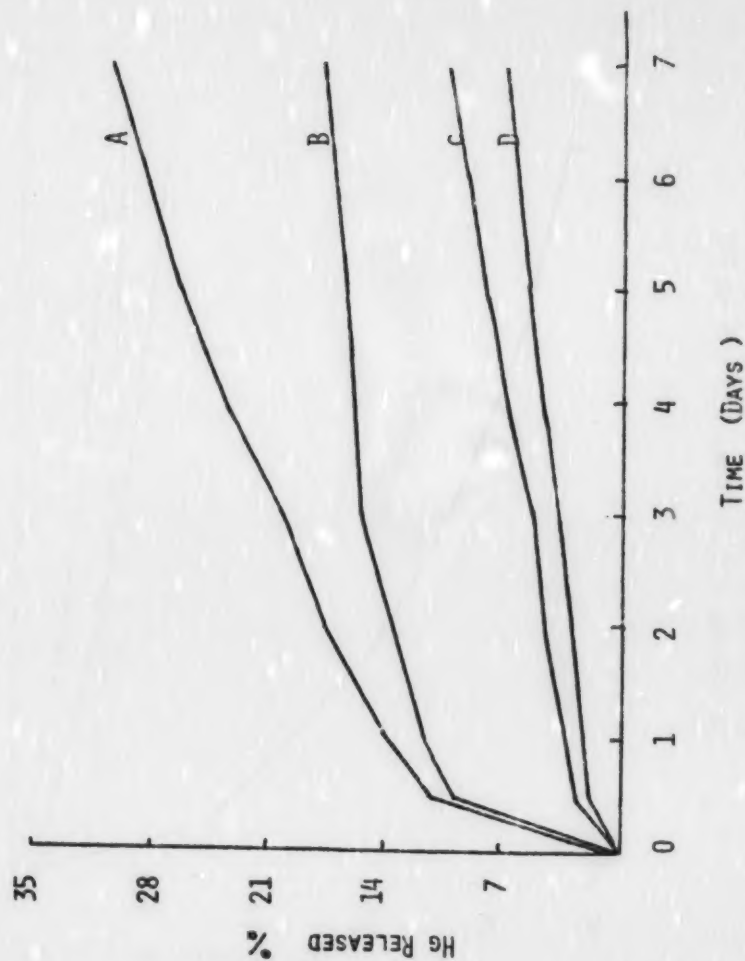


Figure 15. Release of Hg⁰ in the presence of purified Clark Hill humic acid

was indeed being reduced in the presence of humic acid. A graph of the percentage of elemental mercury released in a week of continuous bubbling with N_2 is presented in Fig. 5. The trend is for decreasing release with increasing pH, which correlates with the previous observation that uptake by humic acid decreases with increasing pH. With this relationship between pH and release in mind, all of the following experiments with pure humic acid were arbitrarily done at approximately pH 7 for consistency of results.

In studying the effect on release of varying concentrations of both mercury and humic acid, it became apparent that the initial ratio between the two was more important than the absolute concentration of either one. For instance, the percentage released as Hg^0 in one week was the same when the flask contained either 2 mg mercury plus 1 mg HA or 0.2 mg mercury and 0.1 mg HA; in both cases the ratio of humic acid to mercury is 0.5 (Fig. 6). Further data on elemental mercury release at different ratios of humic acid to mercury are presented in Figures 7-10 and summarized in Fig. 11. When the ratio is low, at 0.5, release of Hg^0 is very slow but continues over the entire time period. Increasing the ratio to five and on up to 100, greatly increases the amount of release, up to a maximum of about 20% in a week. Further increase until there is a large excess of humic acid again decreases the rate of release and actually almost stops it after 3 to 4 days. This same pattern holds true when humic acid is added, i.e., the HA:Hg ratio is increased, during the course of a reaction (Fig. 12). Increasing the ratio from 0.5 to 1 slightly increases the rate of release, while an increase from 5 to 10 greatly speeds the rate of release. The increase from 43.5 to 87 produces a very slight increase, while adding more humic acid to a system where it is already in excess (the increase from 400 to 800) has no effect. When there is no humic acid present, release amounts to an average of about 1.5 micrograms of mercury per week (10 to 200 micrograms added mercury) and has no apparent relationship to the pattern described above. In fact, the addition of humic acid can decrease the amount of release below 1.5 μg (Fig. 11).

All of the preceding experiments concerning the effect of the HA:Hg ratio on elemental mercury release were done using humic acid purified from Lago Pond sediments, so it was necessary to test humic acids isolated from other sediments. The results from three different humic acids (Par Pond, Lake Hartwell and Clark Hill) are presented in Figures 13 through 15, and the same pattern of release is evident in all three cases, i.e. lower release at the extreme ratios and higher release in the middle ratios. These differ somewhat from each other and from Lago Pond humic acid, however, in the actual percentage released over one week, particularly the Clark Hill humic acid, which continued to release a large percentage even at large excesses of humic acid.

The possible influence of other factors on reduction and release of mercury was investigated using Lago Pond humic acid. The most notable result was produced by the addition of 10 mg sodium sulfide, Na_2S , to the normal reaction mixture of mercury and humic acid. The reaction was stopped completely, most likely due to the formation of insoluble and unreactive mercuric sulfide, HgS . Substituting 10 mg labeled solid HgS for the soluble mercury usually used had the same effect; there was no release of elemental mercury. Ten mg of certain arbitrarily selected cations, including Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Fe^{3+} and Al^{3+} , were added to various reaction mixtures without any effect on the rate of

release, and bubbling with air instead of N_2 was also without any effect.

The successful reduction of mercury by isolated humic acid led to the following attempts to demonstrate the release of elemental mercury from whole sediments by the action of natural humic acid. Artificially contaminated sedimentary systems were created by adding labeled inorganic mercury to sediments from both Lago Pond and Clark Hill. No purified humic acid was added. Ten g (wet wt.) of Lago Pond sediment or 3 g (wet wt.) of Clark Hill sediment were used. The Eh of both sediments was negative and the pH of the reaction was about 3. The organic content of muds was determined from the data in Table 1 and assumed to be primarily humic material (Schnitzer and Khan, 1972). Therefore, the amounts of added humic acid were approximately 300 milligrams for Lago Pond sediments and 100 milligrams for Clark Hill. The release of Hg^0 again was verified by cysteine trapping.

In a first set of experiments, ten μg of mercury were added to Lago Pond sediment, as in the initial uptake experiments. Uptake of mercury by the sediment was total and continuous incubation failed to release any mercury over three weeks. Since there was a large excess of humic acid in the sediments over the amount of mercury added, which could be inhibiting release, another series of experiments was done with very large amounts of added mercury in an attempt to decrease the HA:Hg ratio. These experiments were parallel to the uptake determinations at very high concentrations of added mercury. The results for both Lago Pond and Clark Hill are presented in Table 4. The rate of release was slow, especially from Lago Pond sediments, producing sharply lower percentages of released elemental mercury after one week. The important point to note, however, is that the pattern of release is the same as that found earlier for pure humic acids. The greatest percentage release is found when there is 20-30 times as much humic acid as mercury. Release is considerably less when there is less humic acid and when there is a large excess. This pattern indicates that the same basic processes are occurring in whole sediments regarding the interaction between humic acid and mercury as was found with purified humic acid.

FISH

Uptake Experiments

Gambusia readily accumulate Hg^0 directly from the water under short term continuous exposure conditions (see Fig. 16). It can be seen that after 24 hours, Gambusia had taken up approximately 20 ppm or 0.83 μg mercury/hours/wet weight fish in a 0.1 ppm total mercury solution. The Hg^0 uptake curve and the $HgCl_2$ uptake are almost identical. Although it may be expected that Hg^0 uptake would be greater than $HgCl_2$, later evidence suggests that apparently physical adsorption processes, rather lipid solubility differences, largely determine the initial rate of uptake. An uptake of approximately 20 ppm $HgCl_2$ by Gambusia after 24 hours in a 0.1 ppm solution agrees quite well with values determined by McKone et al., (1971) for goldfish, Carrassium auratus. Goldfish take up approximately 22 ppm $HgCl_2$, measured as total mercury, after 24 hours in a 0.25 ppm solution. Both the Hg^0 and $HgCl_2$ curves indicate a tendency to level off with time which suggests that an equilibrium between the fish and

TABLE 4. RELEASE OF Hg^0 FROM LAGO POND (10 g WET WT) AND CLARK HILL (3 g WET WT) SEDIMENTS ($E_h = -100$ mv, $\text{pH} = 3.0$)

Source of Sediment and Mercury added (mg)	% release as Hg^0 in 168 hours
Lago Pond*:	
1	0
2	0
5	0.03
10	0.33
50	0.19
100	0.04
500	0.02
1000	0
Clark Hill:	
0.01	0.03
0.1	2.87
0.5	7.52
1	11.23
2	10.39
5	13.75
10	1.94
50	2.62
100	4.75
500	1.23

* Average of two determinations

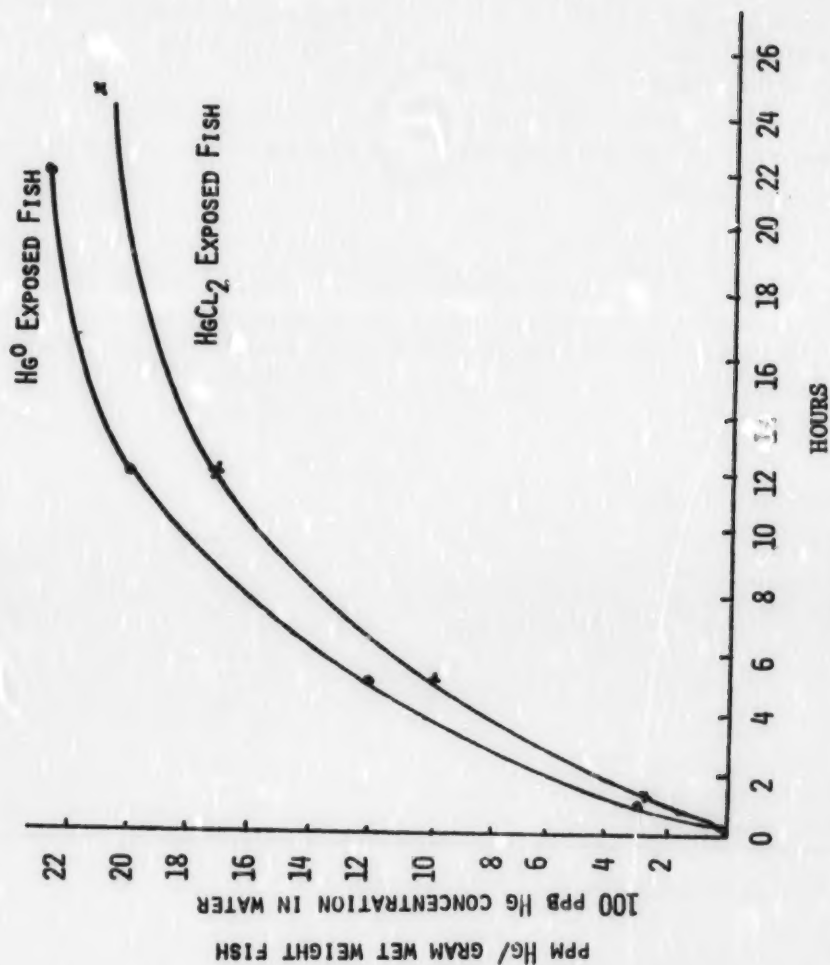


Figure 16. Uptake of Hg^0 and HgCl_2 by *Gambusia* under continuous exposure conditions. Each point represents the average of 3 fish. Values corrected for background concentration of 0.22 ppm Hg

the water would be reached at some point in time. However, the time period of this experiment was not long enough to reach that point. Since the uptake by Gambusia was so rapid and reached such high concentrations within a short time period, it was of interest to see just how much was actually being incorporated into the fish and how much was merely sorbed to the epidermal mucous secretions. Comparisons between scraped and unscraped Gambusia indicated that 13 to 19% of the total mercury is sorbed onto the outside of the fish. Since mere rinsing of the body is not expected to remove much of the mucous on the gills, the percent adsorption is considered a quite conservative estimate. The concentration of mercury in the external mucous of fish has also been noted for inorganic mercury (McKone et al., 1971) and methylmercury (Burrows and Krenkel, 1973).

Under conditions of periodic exposure of Gambusia to ~50 to 75 ppb solutions of Hg^0 and HgCl_2 , a somewhat different picture is seen from the curves in Figure 17, as compared with Figure 16. Although the sample size is small in this experiment, 1 fish per point, it can clearly be seen that Hg^0 is taken up more readily than HgCl_2 , about 5 times greater. This difference is explainable in terms of the physical properties of the compounds. Hg^0 is uncharged and its high lipid solubility enable it to penetrate the gill membranes faster than HgCl_2 . The bivalency of HgCl_2 would cause it to bind tighter to the mucoproteins of the gills and thus its diffusion into the gills would be restricted (Olson et al., 1973). Once Hg^0 is dissolved in the blood, it is rapidly oxidized to the mercuric ion and is distributed and metabolized accordingly (Nordberg and Skerfving, 1972). Mucus-sorbed Hg^0 and HgCl_2 is sloughed off quite easily during the interim holding periods which is presumed to be why the maximum body burden of these mercury species under these periodic conditions is less than that reached under continuous exposure conditions.

Elimination

The elimination of mercury by fish exposed to both Hg^0 and HgCl_2 for 2-hour exposure periods and then held in mercury-free water for varying time periods can also be seen in Figure 17. It can be seen that the slopes of the two curves were still positive until exposure ceased after 10 days, indicating that after the 10 2-hour exposures, an equilibrium between the fish and water concentration had not been reached. The curves also show that Hg^0 is eliminated by Gambusia at approximately the same rate as HgCl_2 . This is to be expected since Hg^0 is eventually oxidized to Hg^{+2} and is distributed and metabolized in the same pattern as Hg^{+2} (Bidstrup, 1964; Kudsk, 1973). The elimination rate appears to be quite rapid for both curves, with the biological half-life approximately equal to 45 days. These data agree with literature values. Goldfish intraperitoneally injected with $^{203}\text{Hg}(\text{NO}_3)_2$ lost mercury at a constant rate resulting in the half-life of 568 hours (~24 days) (Weisbart, 1973). Miettinen et al. (1968) found in some marine and freshwater organisms that the biological half-life on mercuric nitrate ranged from 10 to 43 days.

Biotransformation

The various experiments on in vivo and in vitro methylation did not prove successful (Table 5). The hypothesis that methylation of inorganic mercury can occur in vitro and in vivo via the methylcobalamin mechanism is strongly

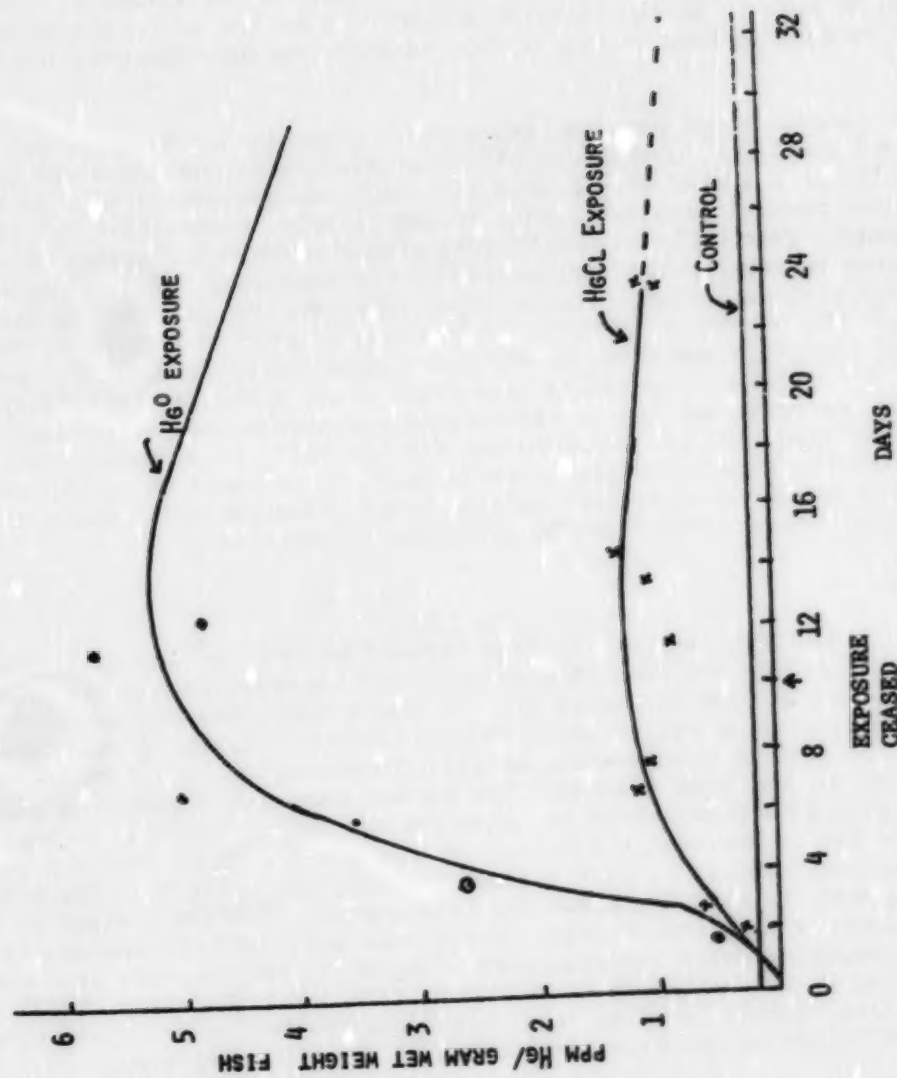


Figure 17. Hg^0 and $HgCl$ uptake by *Gambusia*; 10-2 hr exposures (100 ppb Hg/day) followed by holding fish in Hg -free water

suggested by the literature. Studies of the chemical methylation of mercury indicate that methylcobalamin is at least partly responsible for the methylation of mercury in nature (Imura et al., 1971; Bertilsson and Neujahr, 1971). Methylcobalamin has been identified not only in microorganisms but also in calf liver, blood plasma and pork liver (Wood et al., 1968; Lindstrand, 1964; Burke et al., 1970). Vitamin B₁₂, of which methylcobalamin is a derivative, is stored in fish largely in the liver (Smith, 1965). Stronger experimental evidence that suggests in vivo methylation can occur in fish can be seen in the work of Imura et al. (1972). He found in vitro methylation by liver homogenates of several tuna species but only to a lesser extent by amber jack, mackerel and rainbow trout. His analytical procedure and sample sizes were replicated in this study using bluegill and trout livers, but his results could not be replicated. Therefore, the inability to find in vivo or in vitro methylation can only be attributed to these factors: 1) the formation of thick emulsions in the extract which resulted in low extraction efficiency for methylmercury, and 2) the fish species used may simply not be particularly efficient at methylation.

TABLE 5. METHYL-Hg CONCENTRATIONS IN GAMBUSIA
IN VIVO METHYLATION

# of Fish	# of Exposures + # Days in Fresh Water	Wet Weight (grams)	CH ₃ -Hg (as Hg) µg/g-ppm
Hg ⁰ Exposed fish			
3	Control	1.98	0.16
1	Control	1.04	0.28
1	4 Exposures	0.63	0.28
3	@ 4 Exposures	2.90	0.14
2	<u>9 Exposures</u>	1.92	0.17
	10 Exposures		
1	10 Exposures + 34 Days in Freshwater	0.94	0.36
1	10 Exposures + 23 Days in Freshwater	1.07	0.27
HgCl ₂ Exposed fish			
2	Control	1.82	0.16
1	Control	0.96	0.23
2	<u>10 Exposures</u>	1.52	0.16
	14 Days Fresh- water		
1	10 Exposures + 13 Days Fresh- water	0.66	0.50
1	10 Exposures + 32 Days Fresh- water	1.04	0.27
2	10 Exposures + 33 Days in Fresh- water	1.10	0.31

SECTION V

DISCUSSION

MERCURY

Uptake of Mercury

The interaction of mercury with humic material is an interesting phenomenon and may be an important component of mercury dynamics in aquatic systems. There is little doubt that sediments as a whole act as both a sink and a source for mercury by absorbing large quantities of input and releasing various transformed species (Fishbein, 1970; Krenkel, 1973). In this study, when moderate amounts of mercury are added to Lago Pond sediment, one of the more striking results is nearly total uptake from the water in a relatively short time interval (Table 2). This is not surprising since mercury can be adsorbed or complexed by a variety of materials under many different conditions. In natural systems, uptake may be slower, depending on the amount of suspended sediments and the settling rate, but the resulting low concentrations in water are similar, usually around 1 ppb or less and often not measurable (D'Itri et al., 1971; Krenkel, 1973). Fulkerson et al. (1974) report dissolved mercury concentrations in a contaminated river-reservoir system to be generally less than 0.05 ppb, and even when sediment concentrations rise above 150 ppm, the concentration in the water does not exceed 0.2 ppb.

The validity of shaking as a means to establish contact between sediments and mercury in the uptake experiments was a concern of ours. There is no doubt that shaking decreases the time necessary for the mercury to reach equilibrium between the water and the sediments; however, the results are the same as those observed naturally, i.e., very little mercury remains in the water (Lindberg and Harris, 1974). The distribution remains similar with respect to the base-extractable organics, also, since 20-30% of the mercury was found in that fraction in both experimental systems and in actual samples from Par Pond (Tables 1 and 2). Since the interstitial water and the organic fraction are of primary interest, the shaking procedure probably had little effect on the results.

Looking at the mercury associated with the extracted organic compounds (Table 2), an appreciable fraction of the total mercury in the sediment is found in the KOH extract, in good agreement with natural amounts measured in Par Pond sediments (Table 1). Among the possible sites of adsorption of mercury on organic molecules are the sulfhydryl ($-SH$) and amine ($-NH_2$) groups and the more numerous carboxyls and other oxygen-containing groups. Uptake by sulfhydryls is much faster (Reimers and Krenkel, 1974) but the numbers of oxygen-

containing groups may make them important. It is easy to see that mercury uptake will depend to some extent on the exact nature of the organic molecule.

Another factor affecting uptake by organics is the negative correlation with pH. This may seem unusual, since hydrogen ions compete with mercury for ligands and should displace mercury at low pH. However, several other processes are occurring to complicate the reaction. One of the most obvious effects of pH on humic acid is the change in configuration, reflected in decreasing solubility at low pH. This change may be able to stabilize the binding of mercury to the humic molecule and decrease the probability of replacement by hydrogen ions. In regard to mercury uptake by both humic and fulvic acid, increasing pH will have the effect of increasing the hydroxyl concentration, which will be another ligand competing for the mercury. There is also increased ionization of HgCl_2 at lower pH, and the mercuric ion can form two ligands with the organic molecules to stabilize itself. These and other mechanisms have been postulated by Webb (1966, p. 760) to explain the increasing reaction of mercurials with proteins at low pH and may be applicable in this case.

The uptake of mercury by fulvic acid is also found to vary several fold in negative proportion to the Eh of the sediment. Among the reasons for this is the possibility that the reducing environment somehow alters the fulvic acid molecule, perhaps by reducing sulfur groups, so that mercury is more strongly bound. Another possibility is that the amount of fulvic acid is increasing, resulting from a breakdown of the larger humic molecules under low redox conditions. There is some evidence for this in a corresponding decrease in the amount of humic acid where fulvic acid uptake is high, resulting in the positive correlation between humic acid uptake and Eh (Table 2).

When very large amounts of mercury are added to sedimentary systems at low Eh and pH, uptake patterns are altered in several ways compared to moderate additions. Most noticeable is the fact that the sediments are not able to continue taking up mercury past a certain amount, and a large proportion remains in the interstitial water. The amounts of the different sediments used were chosen to provide roughly equal dry weights, and they were found to "overload" at approximately the same amount of added mercury, i.e., between 10-50 mg. The break between low and high percentages of mercury in the interstitial water is more gradual in the Clark Hill sediments, an indication of some differences between the two sediments. In normal situations these extremely high concentrations of mercury (greater than 5000 ppm) will rarely be reached. Looking at uptake by humic acid, in both sediments the percentage of mercury adsorbed apparently begins to decline after more than one mg. has been added (Tables 2 and 3). In other words, the ability of humic acid to take up mercury is limited in these sediments. The fulvic acid, however, continues to increase its share of the added mercury up to almost ten mg before it begins to decline. The difference between the two sediments is again noticeable, particularly the greater uptake by the fulvic acid of Clark Hill.

The substantial uptake of mercury by organic material in the sediment provides a large reservoir for possible release as elemental mercury. The use of purified humic acid is necessary to simplify the reaction and prove that humic material can reduce mercury. Of course, the purification process greatly alters the humic molecule from its natural state (Dormaar et al., 1970). These

changes could include breakdown of the molecule into smaller organic molecules or loss of organic material altogether, changes in the charge on the molecule, and loss of complexed metals and clays. The presence of acid-soluble material in pure humic acid preparations indicated that some breakdown was occurring, so that the organic material used in the reduction experiments was actually a mixture of both humic and fulvic acids. The following discussion refers to this mixture as humic material. The actual mechanism of the reduction process is unknown but involves the formation of some kind of humic-mercury complex which will allow the transfer of electrons to the mercury. The source of the electrons could be the organic molecule itself, in which case it becomes oxidized, or there could be a transfer of electrons by the humic material from some other electron donor.

The behavior of humic materials in oxidation-reduction reactions has led to speculation on the possible dynamic electron donor-acceptor role of these compounds in nature (Steelink and Tollin 1967, Ziehmman 1972, Schindler and Alberts 1974). If humic materials are capable of serving as electron donor-acceptors in anoxic sedimentary environments such as the profundal regions of lakes, they may be significant in directing the biogeochemical reactions of the system.

Electron transport in organic macromolecules may involve the formation of semiquinone intermediates which may be stabilized through various resonance structures and electron sharing with metals; the quinone configurations which are characteristic of humic materials suggests that their redox reaction mechanisms could also involve semi-quinone intermediates, perhaps also stabilized by metals.

Actual transport by humic substances was demonstrated with experiments utilizing INT [2-p-Iodophenyl-3-p-nitrophenyl-5-phenyl-tetrazolium chloride]. The technique involves the reduction of tetrazolium to formazan by interaction with the electron transport assemblage (flavoproteins, quinones and cytochromes) and substitution of INT for the normal terminal electron acceptor. Since humic acids apparently display a reversible quinone-semiquinone configuration, tests were conducted to determine if INT was capable of serving as an artificial acceptor for humic molecules. Specifics of tetrazolium preparation and formazan extraction and determination have been reported previously (Zimmerman 1974).

After determining that humic-tetrazolium electron transfers do occur, and that humics themselves do not contain a significant number of transferable electrons, various donors (SnCl_2 , NaBH_4 , ascorbic acid, $\text{C}_2\text{H}_5\text{OH}$) were chosen to test the transporting capacity of the humic material. Although all reducing agents eventually reduced INT in the presence of humic acid, ascorbic acid was chosen as the most satisfactory reducing agent. Figure 18 illustrates the typical responses of INT to reduction by ascorbic acid. The figure demonstrates the rate of the reaction with and without the humic material (as indicated by visible spectroscopy) before the experiments. The faster transfer rates after excess reducing agent was eliminated presumably reflects the effect of reducing various functional groups within the humic molecule.

Additional evidence of transporting capacity was obtained from experiments utilizing ^{203}Hg as a terminal electron acceptor. In this case the mercury was

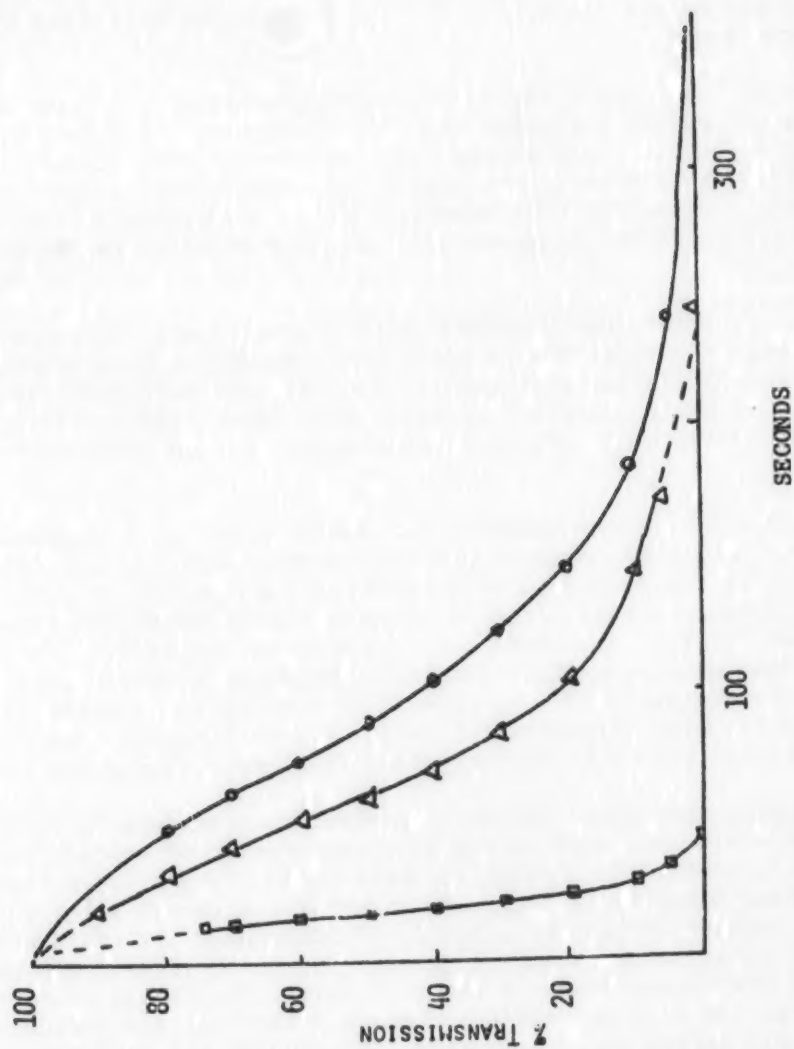


Figure 18. Rates of reduction of INT by Ascorbic Acid, Ascorbic Acid with oxidized Humic Acid, and Ascorbic Acid with reduced Humic Acid
 Ascorbic Acid (2 ml)
 Ascorbic Acid with oxidized Humic Acid
 Ascorbic Acid with reduced Humic Acid

reduced from the ionic form Hg^{++} to the volatile, elemental form (Hg^0). Any elemental mercury formed during the reaction was swept from the system with nitrogen gas, trapped in acid permanganate and monitored with an Ortec Gamma scintillation counter (Alberts, et al. 1974). Initial results of these experiments with chemical reducing agents were identical to the INT tests, suggesting that humic materials facilitate Hg reduction by ascorbic acid. However, these tests with chemical reducing agents only demonstrate the capacity of the humic molecule to transport electrons and do not demonstrate any possible biological significance.

The existence of a humic acid electron pathway could allow anaerobic organisms to utilize a wider range of electron acceptors and continue metabolic oxidations even in the absence of their preferred (or reduction potential necessitated) terminal acceptor. Evidence exists for the possibility that terminal electron acceptors for sulfate reducers in freshwater systems may be limiting (Ramm and Bella 1974). Biologically, a humic acid pathway could allow continued or alternative activity of this particular group which may ultimately result in lessened heavy-metal sulfide deposition. Similar limitations with concomitant biogeochemical consequences could be postulated for other microbial groups.

Consequently, experiments were designed with biological systems to test the ability of organisms to utilize a humic transport system. Initially, whole sediment systems were set up with ^{203}Hg as the electron acceptor. Two levels of mercury were used with three levels of sucrose as a carbon source. Increased release of elemental mercury relative to controls suggests that the organisms were able to reduce mercury in some manner. Progressive carbon stimulation occurred only at the higher of the two mercury levels indicating that the systems were terminal electron acceptor limited rather than carbon limited.

Figure 19 demonstrates a reconstruction experiment using combinations of inocula from anoxic sediment systems, pure humic acids, sucrose and mercury. The results indicate that the organisms are only capable of reducing mercury in the presence of humic acid. Curve C represents the control for abiotic mercury reduction by humic acid (Miller et al., 1974), while the difference between C and D represents additional mercury reduction by organism activity. The experiments do not discriminate between direct utilization of humic acid as an intermediate electron acceptor and indirect transport of electrons from microbially generated reduced substances. However, the net environmental consequence would be the apparent utilization of cationic terminal electron acceptors and a failure to develop pools of reduced byproducts from microbial metabolism. The significance of such alternative metabolic pathways obviously depends upon whether the organisms utilize extracellular transport only in acceptor limited situations or simultaneously with normal metabolism.

Although part of the mercury in both the biological and chemical transport experiments was recovered as the elemental form, some remained in the reaction flask. Extraction and gas chromatographic analysis of this organomercury residual by the methods of Longbottom et al. (1972) lead to the conclusion that an alkylmercury was formed during the transport process (Fig. 20). However, humics could disproportionate as metastable semiquinones and the reactions of mercury with the aromatic moieties on the humic molecule could lead

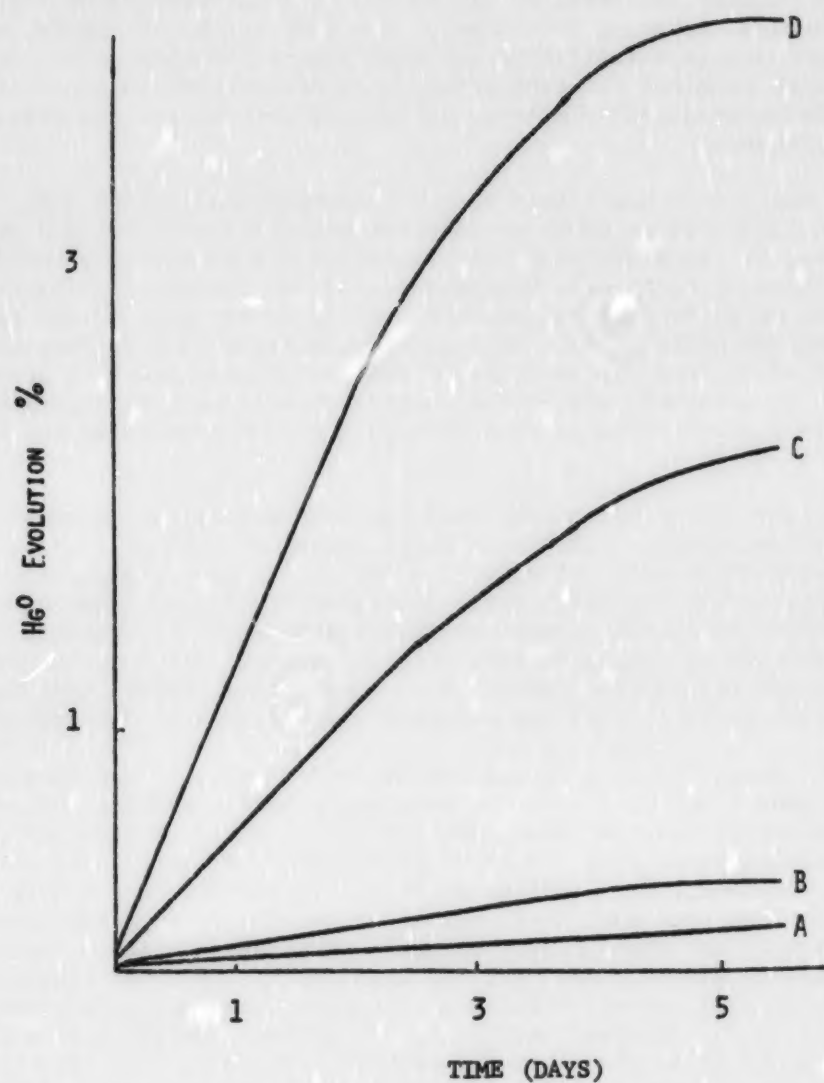


Figure 19. Average percent mercury evolution from four sets of aqueous systems containing anoxic sediment inocula

- A. Mercury
- B. Mercury and Carbon
- C. Mercury and Humic Acid
- D. Mercury, Carbon, Humic Acid

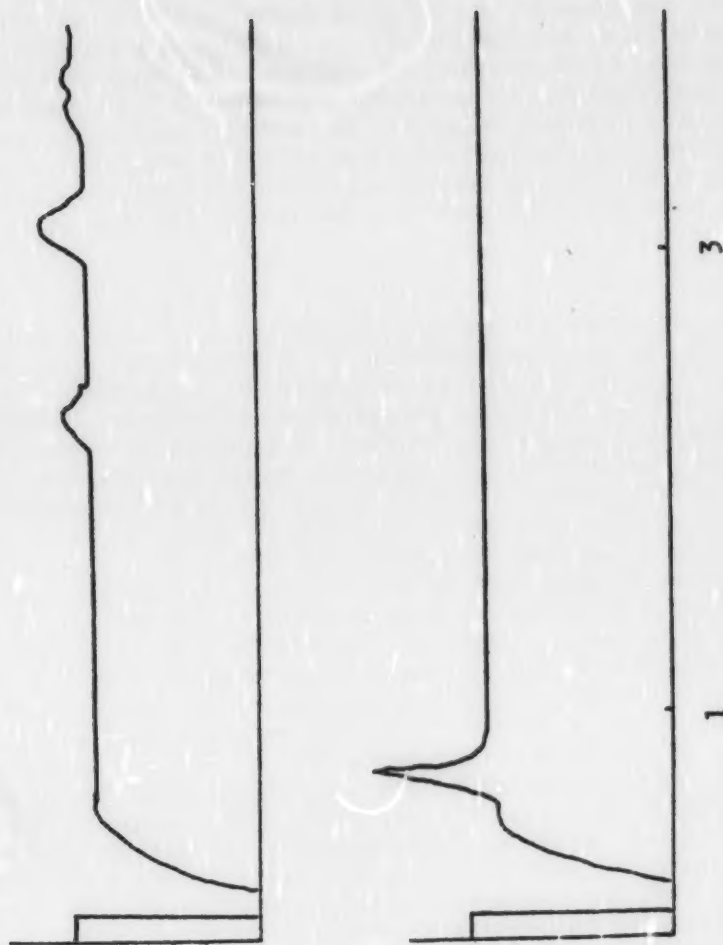


Figure 20. Gas Chromatograph tracings of a 150° isothermal operation yielding one peak with combined CH_3HgI and $\text{C}_6\text{H}_5\text{HgI}$ and a programmed operation from 100° to 160° with an initial hold and a $24^{\circ}/\text{min}$. Rise time yielding CH_3HgI at 2 min 17 sec and $\text{C}_6\text{H}_5\text{HgI}$ at 3 min 10 sec

lead to the formation of aryl derivatives. Therefore, we examined the Longbottom method for its ability to separate alkyl- and aryl-mercury compounds. We concluded that the method was incapable of separating phenyl- and methyl mercury compounds when the gas chromatograph is operated isothermally. However, separations were achieved by operation of the gas chromatograph with a temperature program.

Utilizing this method we concluded that the mercury compound formed in the electron transport reaction of the humic acid was an aryl derivative. Since arylmercury derivatives are apparently less soluble than methyl derivatives in benzene, the Longbottom method could seriously underestimate the amount of organomercury compounds in environmental samples. Furthermore, the dissociation of a complex aromatic molecule like a humic acid would probably yield a family of arylmercury derivatives, most of which would not be extracted or chromatographed following the current isothermal technique. The rates of any of these reactions in nature would depend upon the biotic activity.

Release of Mercury

The rate and the extent of the release of mercury is influenced mostly by the ratio of the two reactants, humic material to mercury (Fig. 10). When that ratio is low, the rate of release is slow from the beginning, so that only a few percent of the total mercury is released over a week. Release is continuing over the entire time period, however, indicating that the reaction is being limited by the number of reduction sites available. As the concentration of humic material is increased relative to the mercury, the reaction speeds up greatly, with a high initial rate of release over the first 24 hours and a slower but continuing release throughout the period of measurement. The maximum amount of release of about 20% in one week is quite substantial and indicates the potential of this process. When the amount of humic material is greatly in excess, however, the reaction is essentially totally inhibited after a small initial quantity of release. A possible explanation for this is that there are actually two types of reactions occurring between the humic material and the mercury, one of which is the reduction process described above, the other a permanent complex formation between the two molecules. This complex could be formed either before or after mercury reduction, since humic acids are known to adsorb Hg^0 vapors (Trost and Bisque, 1972). The result is that all of the mercury is complexed by the excess humic material, totally stopping release. The same pattern of release was found for humic material isolated from other sediments (Figs. 13-15) but the percentages are somewhat different, indicating that the process may be universal, although humic materials vary in the different sediments.

Other factors affecting the reduction of mercury by humic materials include pH and the presence of sulfide, while bubbling with air and adding other cations had no effect. The addition of competing cations was done with the idea of displacing mercury from the humic molecule and increasing release. Strohal and Huljev (1971) tried the same approach, also without success, demonstrating the great stability of the mercury-humic complex. The effect of pH on this reaction is probably due to the increased association between mercury and humic materials at lower pH. Air was used in an attempt to change the redox conditions, which were also found to influence mercury-organic interactions

in uptake experiments, but there was no effect. The purified humic materials are not surprising. The inhibiting effect of added sulfide is predictable and an indication that mercury movement in aquatic systems will be stopped by very low redox conditions leading to the formation of H_2S .

When whole sediments are substituted for purified humic material the complexity of the system is greatly increased, and the results are open to further speculation. The failure of moderately contaminated sediments to release any elemental mercury is most directly attributable to the very large excess of organic material present. When more mercury is added to the same amount of sediment, thereby decreasing the ratio of humic acid to mercury, the same pattern of release as that found previously becomes apparent. Comparing the amount of release (Table 4) with uptake under similar conditions (Table 3) gives some insight into the reasons for this pattern. There is a positive correlation between the amount of mercury taken up by the fulvic acid fraction and the amount of mercury released. This correlation is weak in the Lago Pond data ($r = 0.44$, 6df) but highly significant for the Clark Hill data ($r = 0.78$, 8 df). This leads to the following hypothesis concerning mercury release. As the amount of mercury is increased relative to humic material, the humic acid fraction becomes saturated, and a larger proportion becomes associated with the fulvic acid. This is evident in the data in Tables 2 and 3, where the amount of sediment (therefore, humic material) is constant; the percentage of mercury associated with the humic acid fraction continuously decreases, while the mercury associated with fulvic acid is increasing up to a point and then decreasing. In other words, at a high ratio of humic material to mercury, most of the organically-bound mercury is complexed by the larger humic acid molecules, but as the ratio drops, the fulvic acid contains an increasing fraction of the mercury, leading to an increase in the rate of release. As the ratio drops further, the proportion of mercury in the fulvic acid begins to drop, producing the slower rate of release noted earlier. This hypothesis treats the larger humic acid molecules as a complexing agent which will preferentially adsorb a certain quantity of mercury. Only after this capacity is exceeded will there be an increase in uptake by the smaller fulvic acid molecules, which are the actual sites of mercury reduction. If this is correct, then increasing the amount of fulvic acid, which may occur under reducing conditions, will increase the release of elemental mercury. This can also help explain the obvious differences between the two sediments in their ability to release mercury. The greater release from Clark Hill is probably due to the greater amount of mercury associated with the fulvic acid fraction of the sediment.

Although the formation of Hg^0 by bacteria has been reported by Spangler et al. (1973) as a result of methylmercury degradation and by Summers and Simon (1972) as a result of direct reduction of Hg^{+2} by bacteria, this phenomenon does not appear to be a factor in the research presented here. The presence of bacteria in the experiments involving purified humic material is very unlikely considering the conditions of extraction and purification from sediments and the conditions of the experiment. In the experiments with whole sediments, however, there was no attempt to eliminate bacteria or other microorganisms. The results seem to indicate, though, that the same basic processes are occurring in both sets of experiments. It is very improbable that bacterial production of Hg^0 would vary with mercury concentration in the same way as that

observed (Table 4). There was also no lag period before Hg^0 was produced, as might be expected for bacteria in uncontaminated sediments.

Proposed Mechanisms for Mercury Transport

The possibility of release and recycling of mercury from humic material has not been considered in previous explanations of the mercury cycle. Gavis and Ferguson (1972) mention organic complexing of mercury and speculate that release of inorganic forms may occur if the organic compound is decomposed. Fagerstrom and Jernelov (1972) confirm the affinity of mercury for organic molecules but state that release of inorganic mercury from these complexes is improbable. Organic complexes of mercury have generally been thought to be a block to the continual cycling of mercury (Jenne, 1970). Most of the work concerning the interaction between mercury and organic materials is reviewed by Krenkel (1974) and deals with uptake phenomena. When mercury release from the sediments is considered, methylmercury is presented as the primary form. The formation of methylmercury is thought to be bacterially mediated, so the biological processes of the sediment have been given the most attention (Wood, 1974). Because of the problems with this approach, outlined in the introduction, this study followed a different course. However, the results from both approaches are not mutually exclusive. In Figure 1, both elemental and methylmercury are shown as being released from the sediments to the water. The rate of this release depends on numerous other factors but primarily on the concentrations of organically bound mercury and free Hg^{+2} .

The amount of free Hg^{+2} in the sediment is usually very small, because of the likely formation of various combined and adsorbed forms. This effectively removes a large amount of mercury from the active mercury cycle. Under normal circumstances, only a very small fraction, less than 1%, remains as soluble interstitial Hg^{+2} . This provides a very small pool of mercury available for methylation, although this quantity could be maintained by equilibrium with other forms of mercury. In addition, the methylation process seems to be very slow; Jensen and Jernelov (1969) found that 0.1% of the mercury added at 100 ppm was converted to methyl form in one week. At lower, more realistic concentrations the yield was even less. Jernelov has estimated that the rate of methylation in a natural system will be on the order of 0.1% of the total mercury in the sediment in one year (Asell, 1973). Further reducing the amount of methylmercury is the process of demethylation. Billen et al. (1974) found that, in laboratory experiments with cultures from natural sediments, about 75% of added organic mercury was degraded to elemental form. Not only does this decrease the amount of methylmercury, but it increases the amount of elemental mercury available for release.

The release of elemental mercury from organic complexes in lake sediments depends on both the amount and chemical nature of the organic components of the sediment. These two parameters will vary with the source of organics (autochthonous or allochthonous) and with the sedimentary environment, both chemical and biological. The possibility exists that organically-bound mercury could remain inert and prevent release of the element back into the cycle. Under some circumstances, however, transformation to Hg^0 can occur. The actual rates of release varied widely in the sediments tested here but are undoubtedly slow under natural conditions. They will vary seasonally with Eh and pH of the

sediments and over the long term with inputs of organic matter and mercury. An estimate that 1% of the total mercury is released as Hg^0 per year is probably not too low and is roughly comparable to the rate of methylmercury release.

Actual transfer of either elemental or methylmercury to fish and other organisms will be further complicated by several factors, including stability of both forms in the water column, contact with the biota and affinity for living tissue. In fish, uptake of both forms is probably mainly through the gills and so will depend broadly on metabolism (Fagerstrom et al., 1974). Schoper (1974) studied elemental mercury uptake by *Gambusia* and found concentrations exceeding 20 ppm after 24 hours in water with 100 ppb Hg^0 . Since there was about one gram of fish per liter of water, this is an uptake of 20% of the mercury in the water per gram of fish. The half-time of elimination is approximately 45 days, similar to that for inorganic mercury. Burrows and Krenkel (1973) report similar data for the uptake of methylmercury by bluegills. Using mercury concentrations of only 0.2-0.3 ppb and 2.2 g of fish per liter, they measured uptake at about 40% over five days, or 18% per g of fish. They found long-term loss to have a half-time of about five months, although other workers have reported half-lives of two years for pike in colder waters (Fagerstrom et al., 1974).

The above measurements indicate that both elemental and methylmercury will be taken up by fish to approximately the same degree, although perhaps not quantitatively, and that methylmercury will tend to accumulate to a greater extent than Hg^0 because of its longer retention time. Coupling this knowledge with an estimated rate of release of mercury from sediments can lead to some insights into the mercury contamination problem. Assuming that 1 hectare of sediments contain 1 ppm of mercury and that the active zone of release of mercury is 1 cm deep, then there is 1.5×10^8 micrograms of mercury available for release (sediment density = 1.5g/cc). If the rate of release is 0.1% of this amount per year, then 1.5×10^5 micrograms of mercury will be released, enough to raise the concentration of mercury in 300 kg of fish and other organisms to 0.5 ppm, assuming 100% uptake from the water. Changes in the rate of release to water and in uptake by fish will alter these figures, but the fact remains that a very low rate of release from moderately contaminated sediments can easily lead to contamination in the biota. If possible food chain accumulation is included, then fish, particularly top predators, will reach even higher concentrations.

The importance of the humic-mercury interaction becomes evident here, in that it tends to keep the mercury actively cycling between fish and sediments. Instead of being an unreactive sink in the mercury cycle, organically complexed mercury becomes a large pool available for recycling. This emphasizes the importance of previously contaminated sediments as a continuing source of mercury and means that the mercury cycle is subject to greater variability than previously supposed. Organic input into an aquatic system has been considered a stimulant to bacterial activity, which could cause more mercury release (Wood, 1974). If the humic-mercury ratio is low, addition of organics could have the same effect. But excessive organic input could succeed in permanently binding mercury, providing that it becomes buried. Within a single aquatic system, the organic character of the sediment can vary, leading to differential rates of mercury release as Hg^0 . This can explain the wide ranging concentrations often

found in fish from the same body of water (Schoper, 1974). It can also help explain seasonal changes in mercury concentrations in fish, depending on the rate at which organic material reaches the bottom (Zeller and Finger, 1971). The exact amount of Hg^0 released from the sediment is almost impossible to quantify, as in the case of methylmercury, because of the very small concentrations involved. Some prediction might be possible, however, by studying the nature of the humic-mercury interaction. Not only is the overall ratio of the two components important, but the distribution of mercury between humic and fulvic fractions has an effect on the process of release. If nearly all the mercury in the organic fraction was associated with the humic acid precipitate, then release would be unlikely; however, a significant proportion of mercury in the fulvic acid extract would indicate the possibility of release. Redox and pH conditions have an effect by inversely influencing the amount of mercury associated with the fulvic acid fraction. More work is necessary in this area because actual release rates of methylmercury or Hg^0 have never been determined in the environment.

CADMIUM AND CHROMIUM SEDIMENTS

Since the mercury responds to redox reactions with humic materials, experiments were designed to test the effects of reduction on the behavior of cadmium and chromium using sediments from Clark Hill (low organic content) and Lago Pond (high organic content). 20 g (wet weight) of sediments from each of the systems was slurried with 1 mg/ml of $\text{CrK}(\text{SO}_4)_2 \cdot 12 \text{H}_2\text{O}$ and $\text{CdCl}_2 \cdot 2.5 \text{H}_2\text{O}$ in 50 ml distilled water. Replicates of air oxidized sediments, reduced sediments (1 g Ascorbic Acid) and high sulfide sediments (1 mg Na_2S) were incubated for 24 hours under nitrogen. Analyses of the concentrations of Cd and Cr in the interstitial water (separated by centrifugation at 14,000 rpm for 20 minutes), the organic fraction (soluble in 0.1 N KOH) and the hydrolyzable components (Hot 6N HCl) of the sediment systems were affected after the incubation period (See methods for Hg).

The results of the experiments (Fig. 21) indicate that the cadmium in the sediments of these two systems was affected by the treatment with ascorbic acid and sulfide. Both systems showed an increase in the Cd in the organic fraction. Lago Pond showed a decrease in the Cd in the interstitial water and an increase in the Cd in the hydrolyzable portion of the sediment. The concentration in the hydrolyzable fraction of the Clark Hill sediments decreased in the reduced sediments and the interstitial water concentration remained essentially constant. In spite of the low K_{sp} of cadmium sulfide the behavior of the system was analogous to the ascorbic acid reduction. Furthermore, since the pH of these unbuffered systems varied with the treatments, more cadmium should have been available in the acidic (pH 5-6) ascorbic acid treated sediment than in the relatively alkaline (pH 7-8) sulfide treated sediments. The analogous responses of the two systems to the reduction treatment indicate that cadmium probably associates with the organic fraction of the sediment.

However, more chromium was released into the interstitial water and more Cr was deposited in the organic fraction of both systems as a result of the ascorbic acid treatment (Fig. 22), the addition of sulfide as a reducing agent resulted in the negligible increase in the interstitial water fraction and a

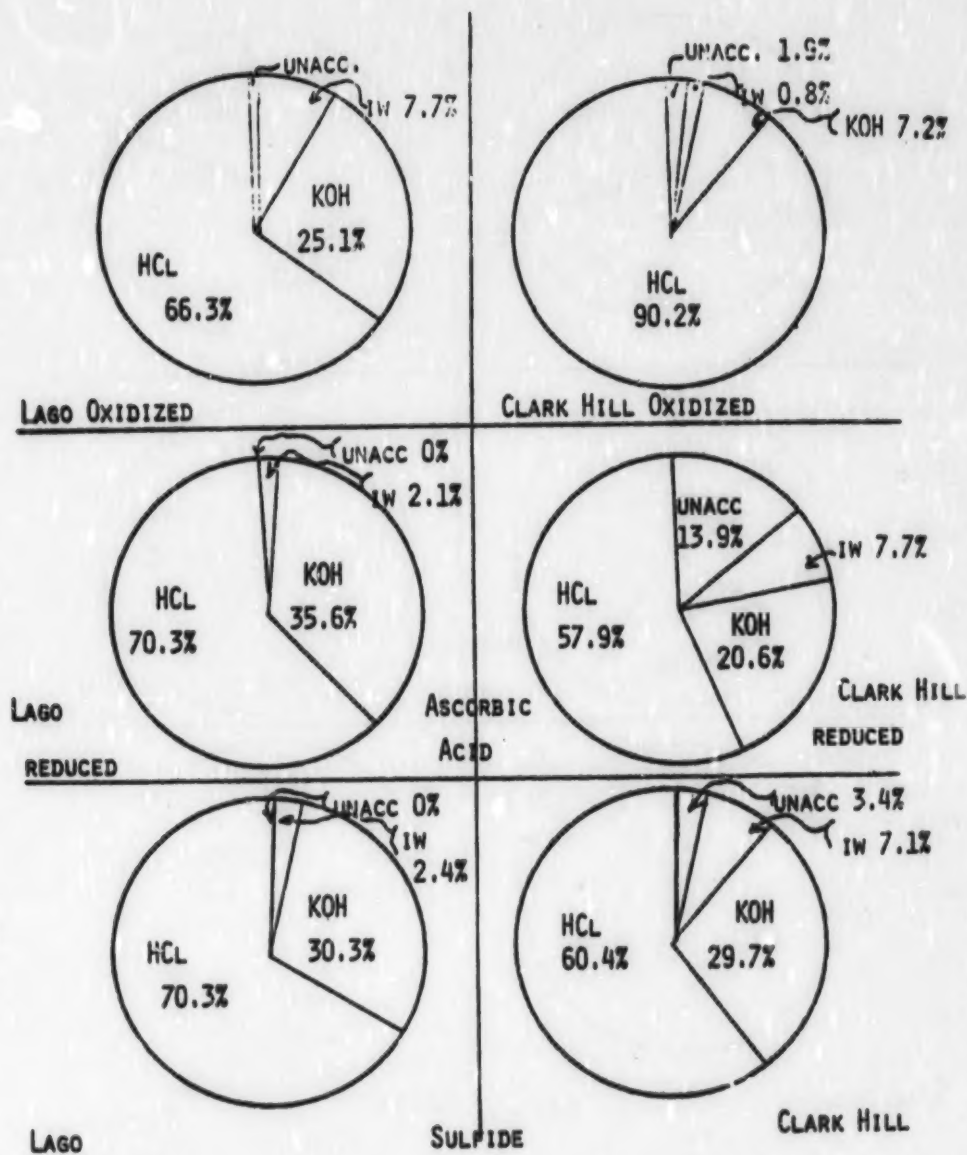


Figure 21. Summary of Cadmium

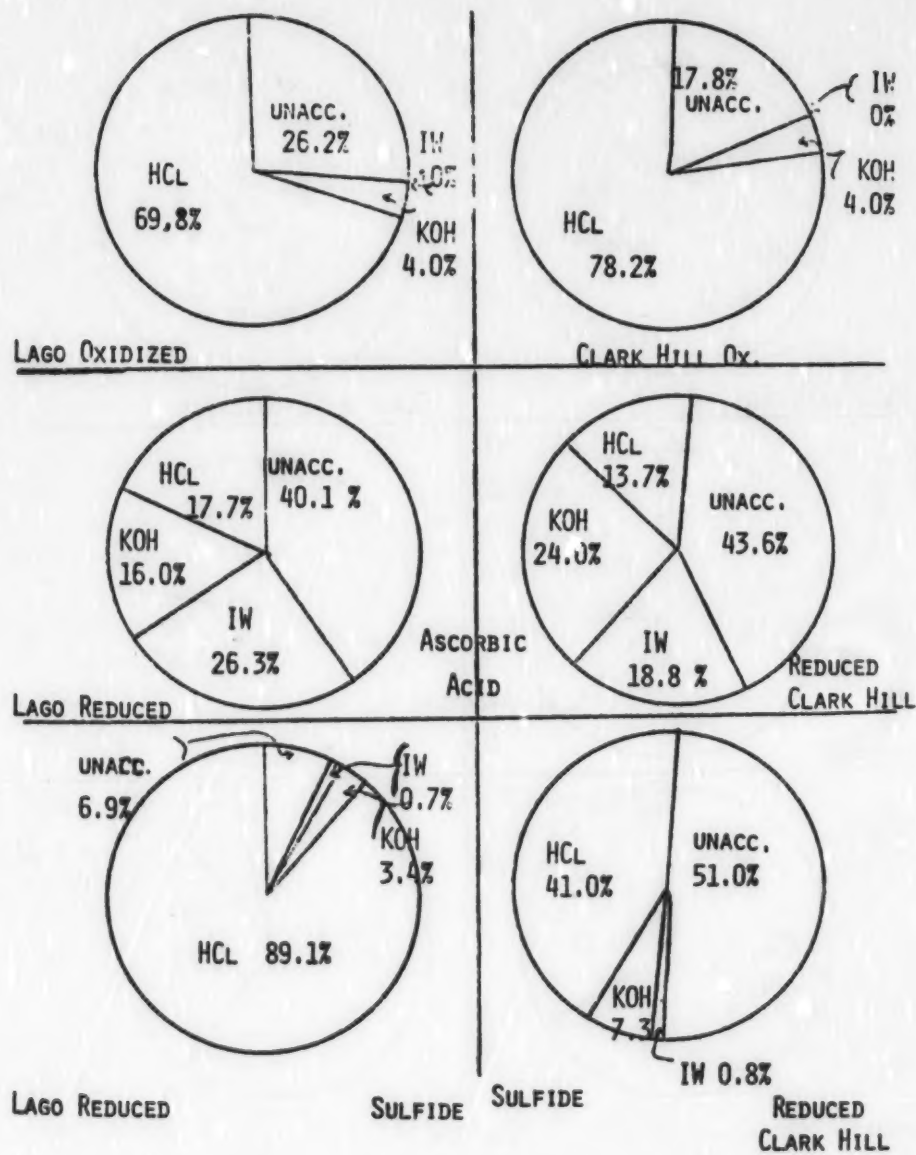


Figure 22. Summary of Chromium

negligible change in the organic fraction. Unlike cadmium, chromium is more likely to form insoluble hydroxides in response to changes in the chemical matrix. These changes may be affected by either the alteration of the pH or alterations of the metal-ligand availability in the system. The responses for Cr in these experiments would be predicted on the basis of the pH alteration of the sediment system.

In spite of the relatively unreactive nature of both Cd and Cr in these cases, the availability of these contaminants to biota will depend upon their free water availability as well as the mechanisms of the food-chain transfer. Since both Cd and Cr have a high affinity for the sediment matrix, transfer to fish could be affected via detritus-eating benthic organisms.

SECTION VI

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